

1 agggagaggc agtgaccatg aaggctgtgc tgcctgccc tngatggca
 51 ggcttggccc tgcagccagg cactgcccig ctgtgctact cctgcaaagc
 101 ccaggtgagc aacgaggact gccctgcaggt ggagaactgc acccagctgg
 151 gggagcagtgc ctggaccgcg cgcatccgcg cagtggcct cctgaccgic
 201 atcagcaaaag gctgcagctt gaactgcgtg gatgactcac aggactacta
 251 cgtgggcaag aagaacatca cgtgctgtga caccgactig tgcaacgccca
 301 gcggggccca tgcctgcag ccggtgcgcg ccacccctgc gctgctccc
 351 gcactcggcc tgcctgctgc gggaccggc cagctatagg ctctgggggg
 401 ccccgctgca gccacacig ggtgtgtgc ccagggcctt tgtgccactc
 451 ctacagaac ctggcccagt gggagcctgt cctggctcct gaggcacatc
 501 ctaacgcaag ttgaccatg tatgtttgca cccctttcc cnaaccctg
 551 acctcccat gggccctttc caggattccn accnggcaga tcagttttag
 601 tganacanat ccgctgcag atggccctc caaccnttn tgttgnigt
 651 tccatggccc agcattttc acccttaacc ctgtgttcag gcactttc
 701 cccaggaag cctccctgc ccacccatt tatgaattga gccaggttg
 751 gtccgtggig tccccgcac ccagcagggg acaggcaatc aggagggccc
 801 agtaaaaggct gagatgaagt ggactgagta gaaatggagg acaagagng
 851 acgtgagnc ctgggagm ccagagatgg ggcctggagg cctggagga
 901 ggggccaggc ctacatig tggggtccc gaatggcagc ctgagcagcag
 951 cgtaggccct taataaacac ctgtggata agccaaaa aa222222

FIGURE 1A

FIGURE 2

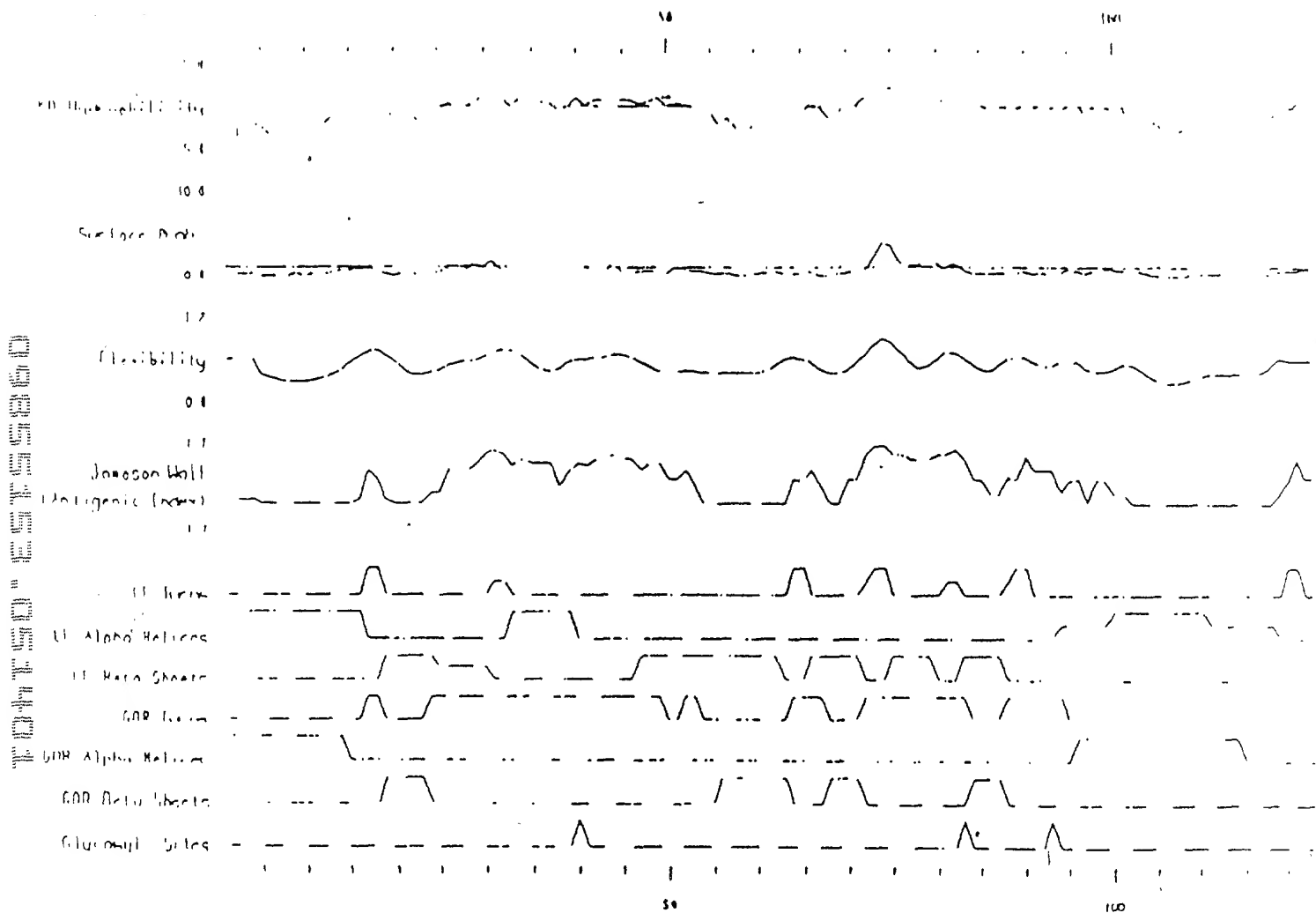


FIGURE 4

↑
Signal
sequence

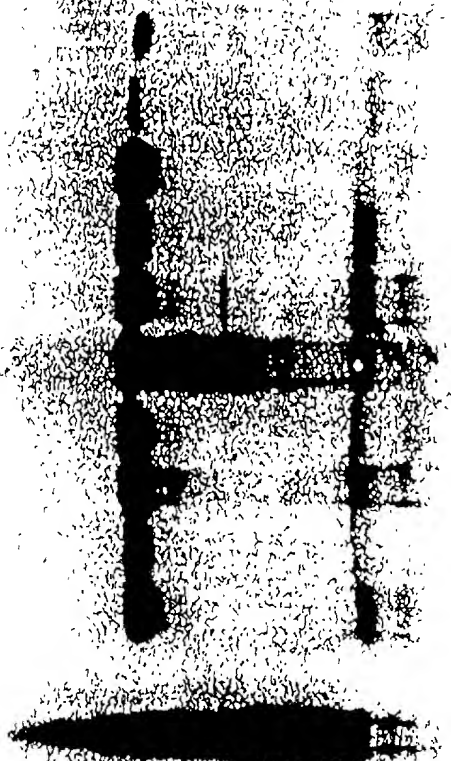
○ = glycosylation
site

✓ GPI signal

FIGURE 5

Western PSCA
 Suppox to be 80mg/ml
 Normal tissue
 1hr exp

1G8
 1:100

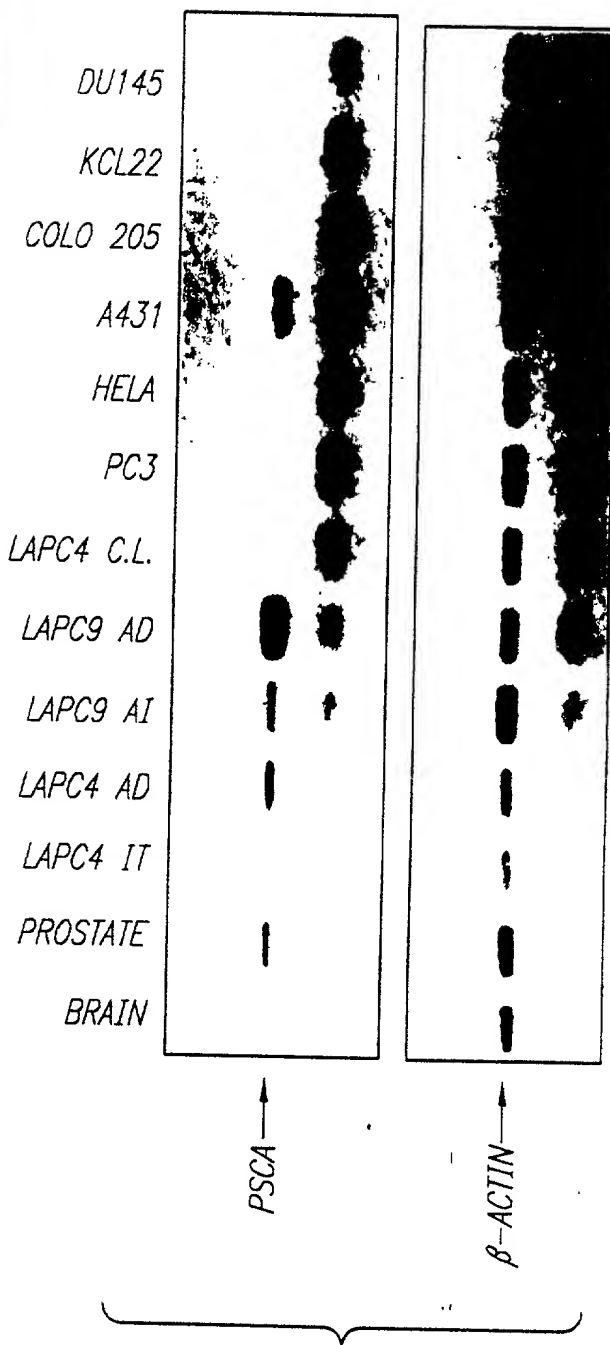
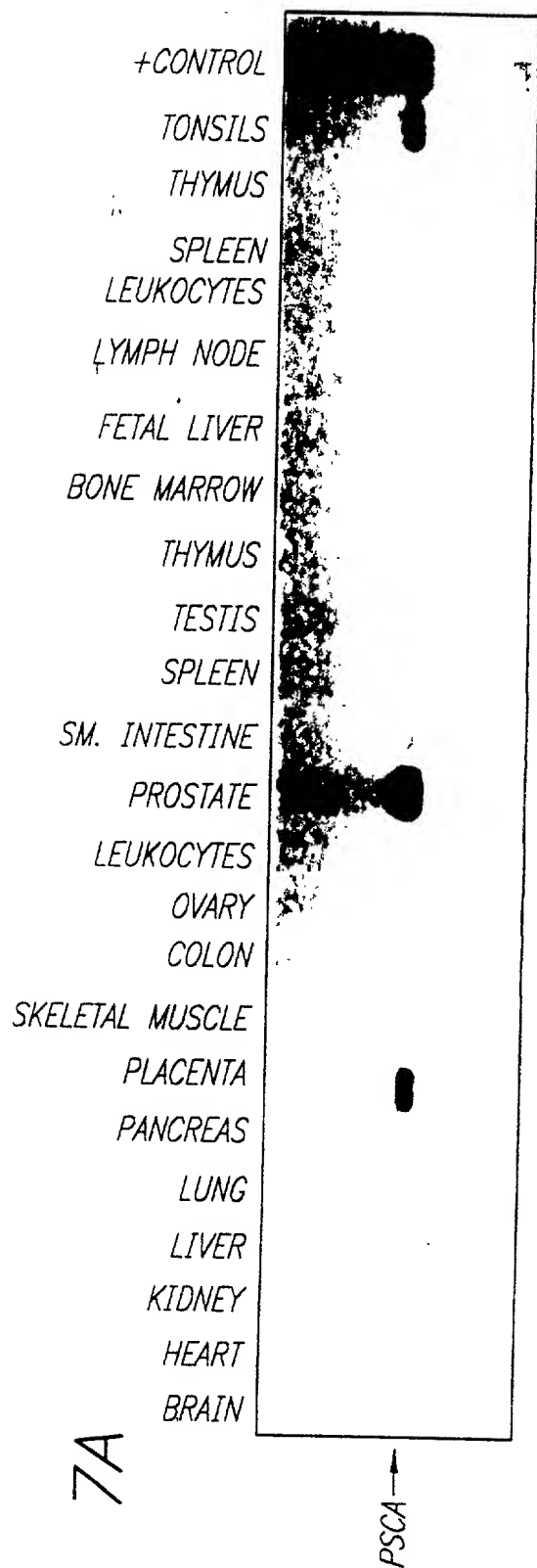




prostate (Kinner)
 prostate (Blake)
 prostate (GCK)
 Bladder (Kinner)
 Bladder (GCK)
 Bladder (Rob)
 Kidney (NABO)
 Kidney (WU2)
 Testis
 Sm. Intest.

LA PC9

09855153.051401

FIGURE 6



Legend:  untranslated region of PSCA
 translated region of PSCA

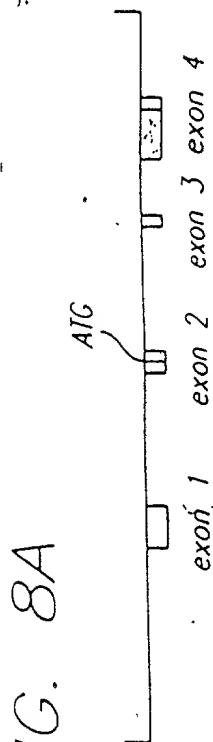


FIG. 8A

FIG. 8B

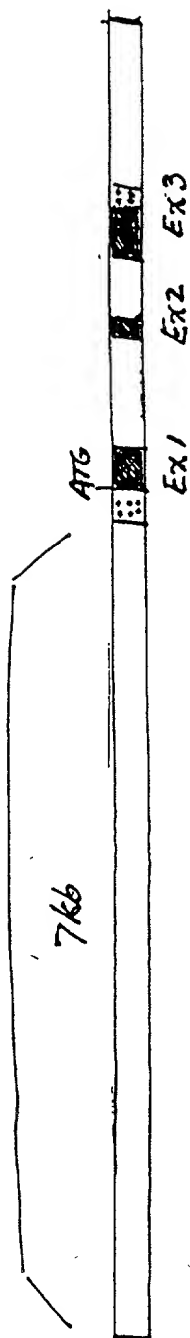
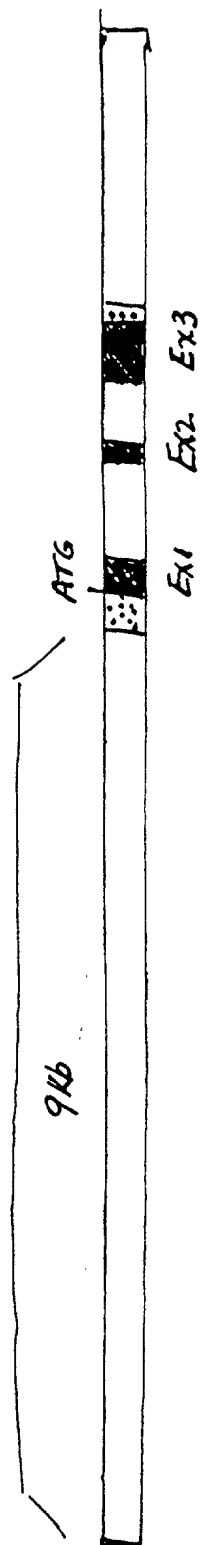


FIG. 8C



human PSCA

FIGURE 8

human PSCA

PSCA / PSA Expression in Benign Prostate vs. Prostate Cancer Xenograft

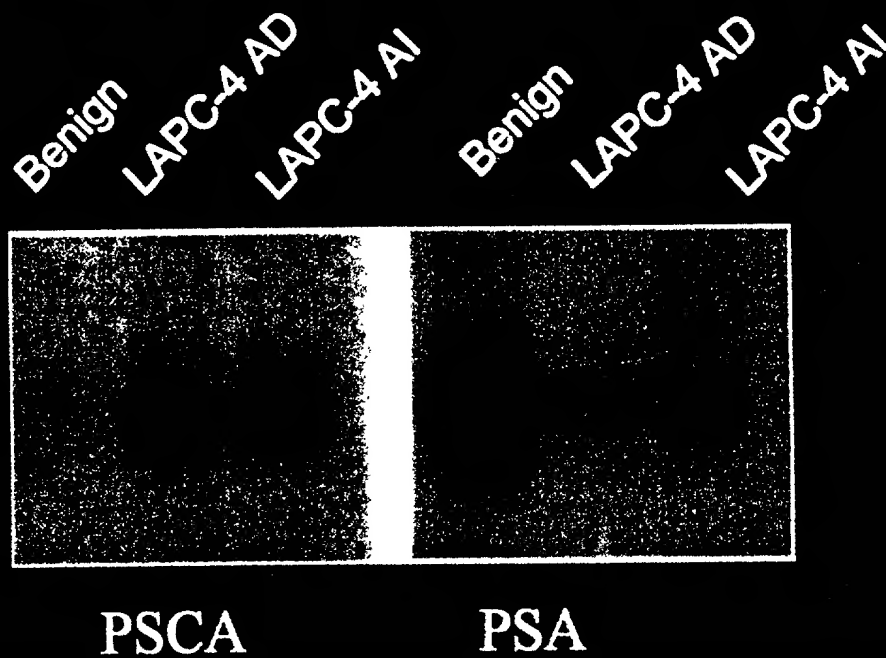
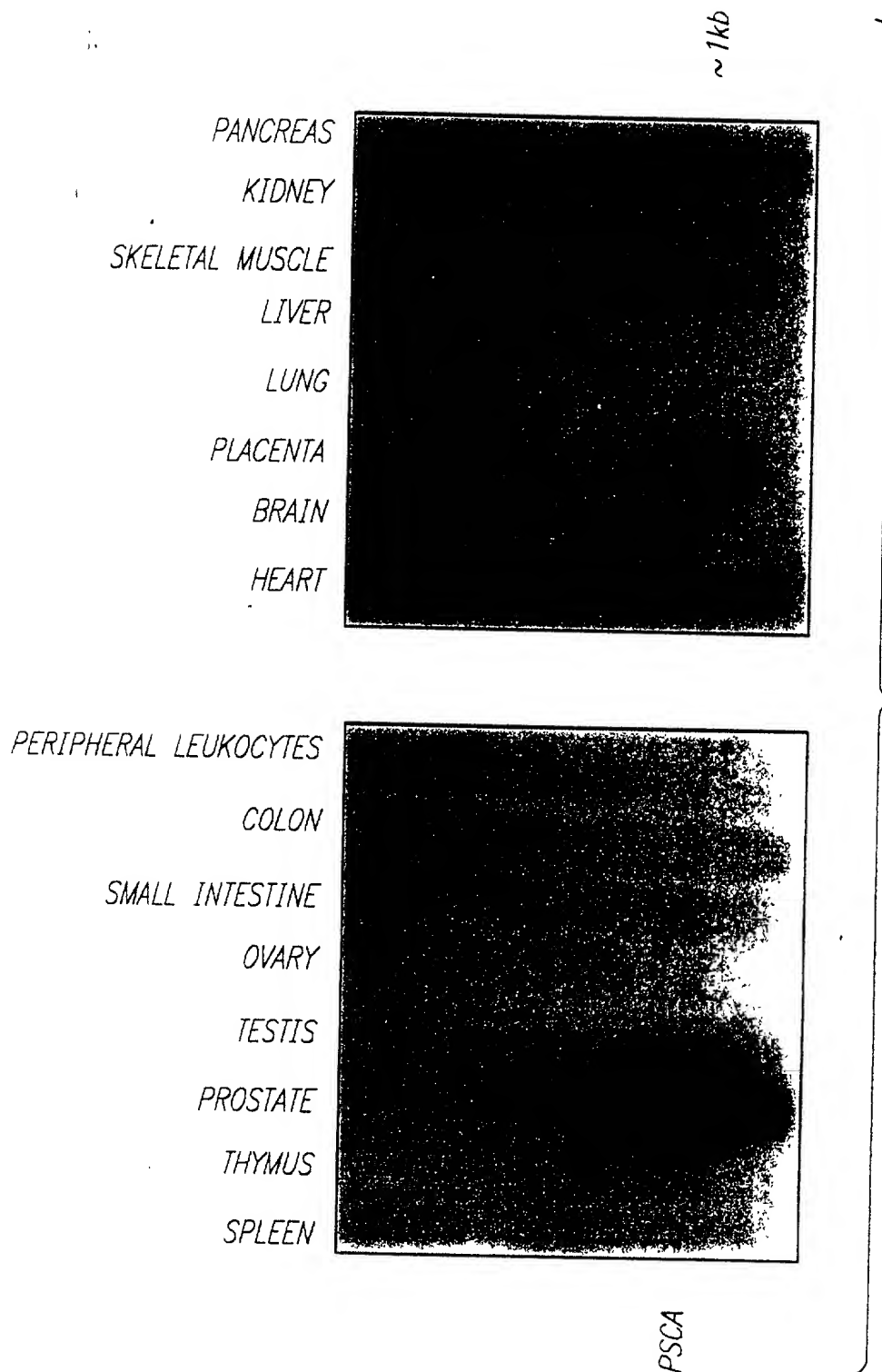
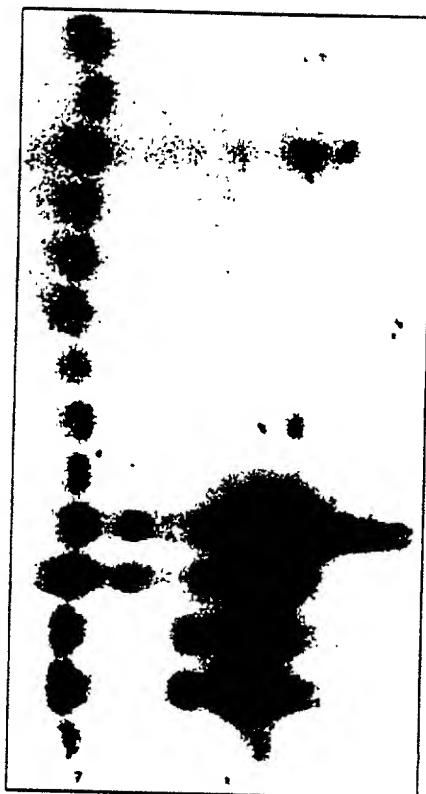


FIGURE 9A



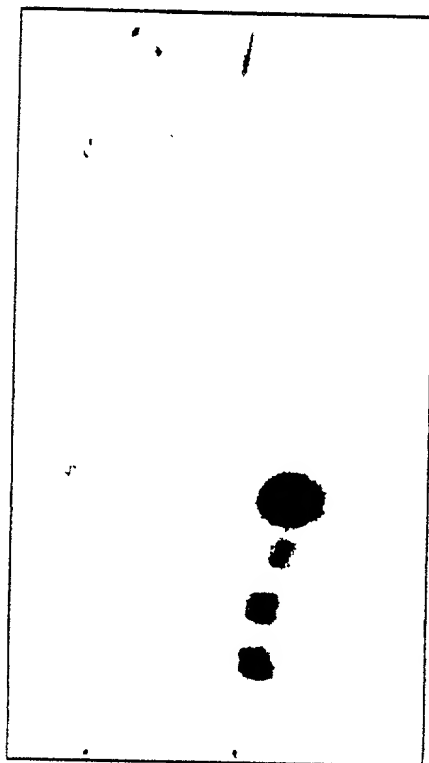
72 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



4 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



PSCA

FIG. 10-1

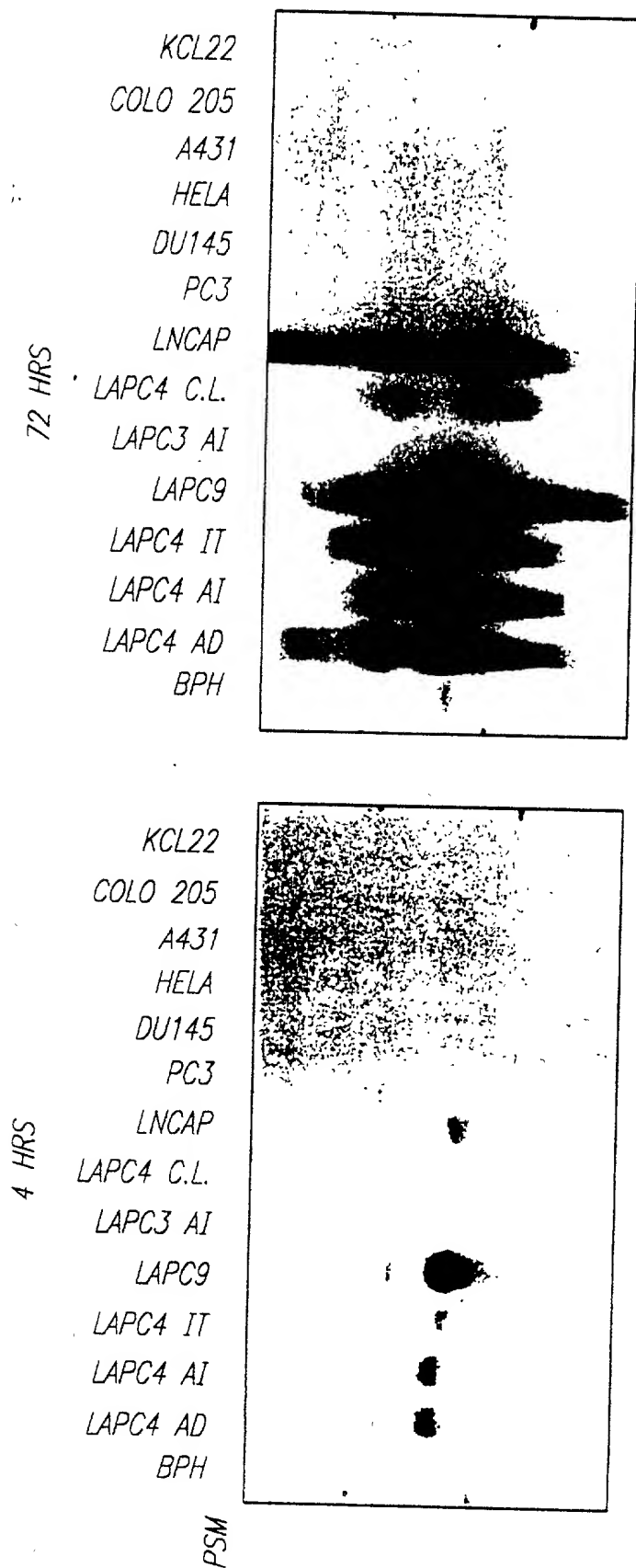


FIG. 10-2

72 HRS

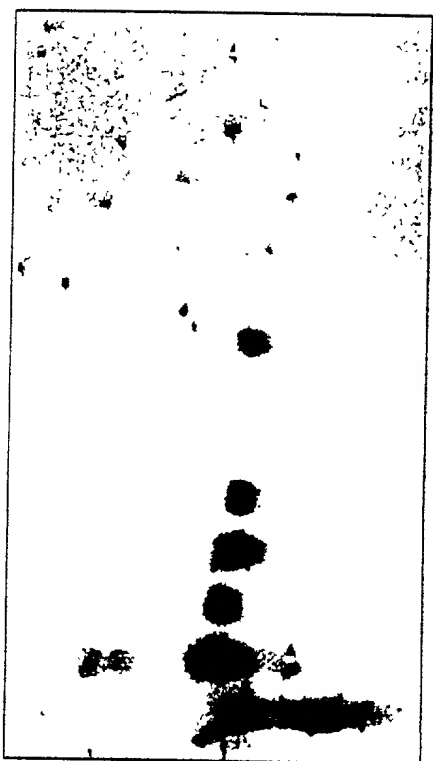
72 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH

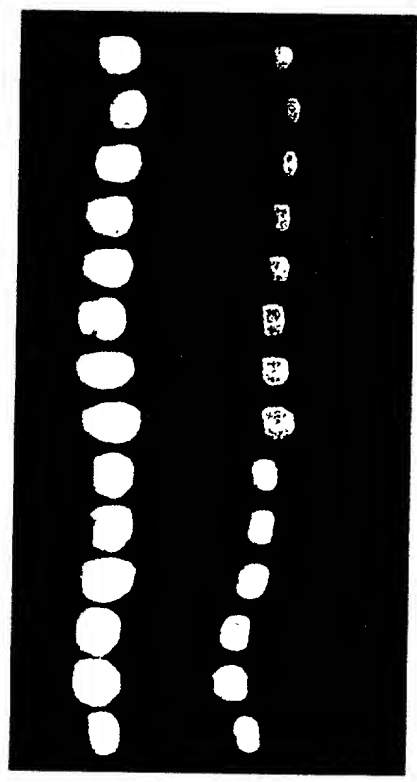


4 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



PSA



EIBR

FIG. 10-3

FIG. 11A

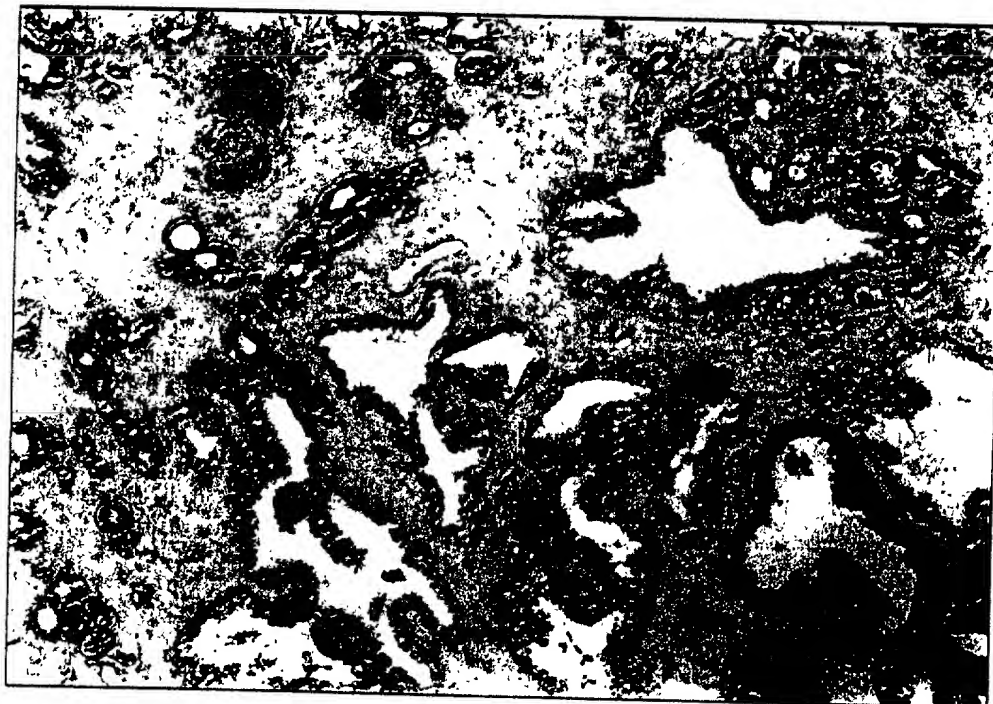


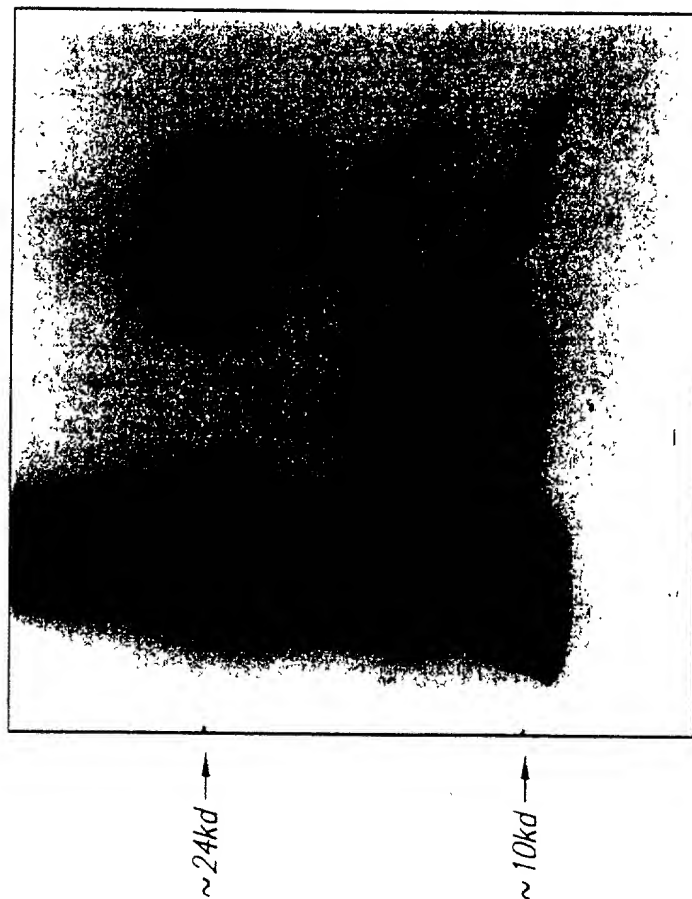
FIG. 11B



FIG. 11C

FIG. 12A

O GLYCOSIDASE
N GLYCOSIDASE F
CONTROL



SECRETED
CELL ASSOCIATED

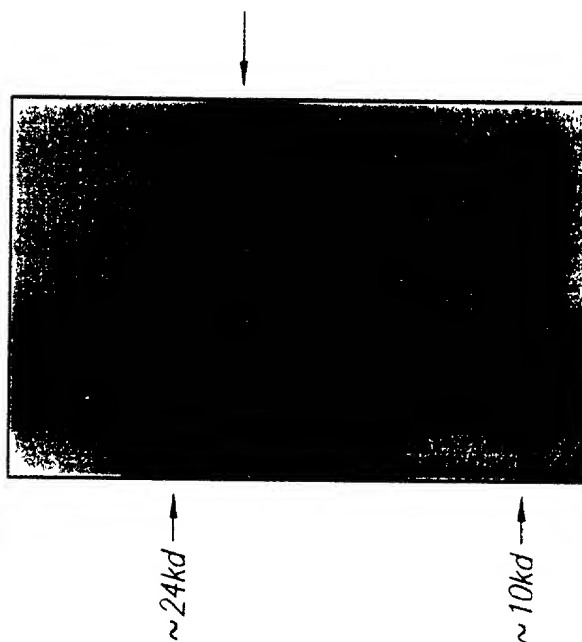


FIG. 12B

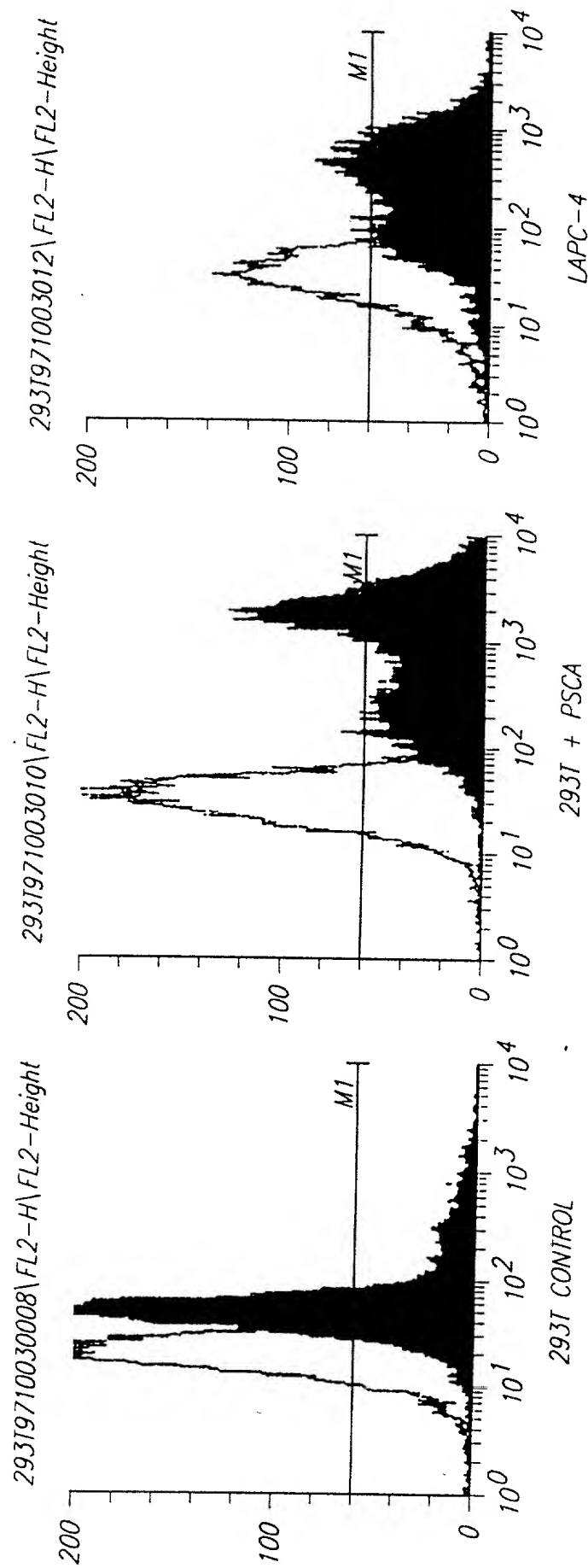
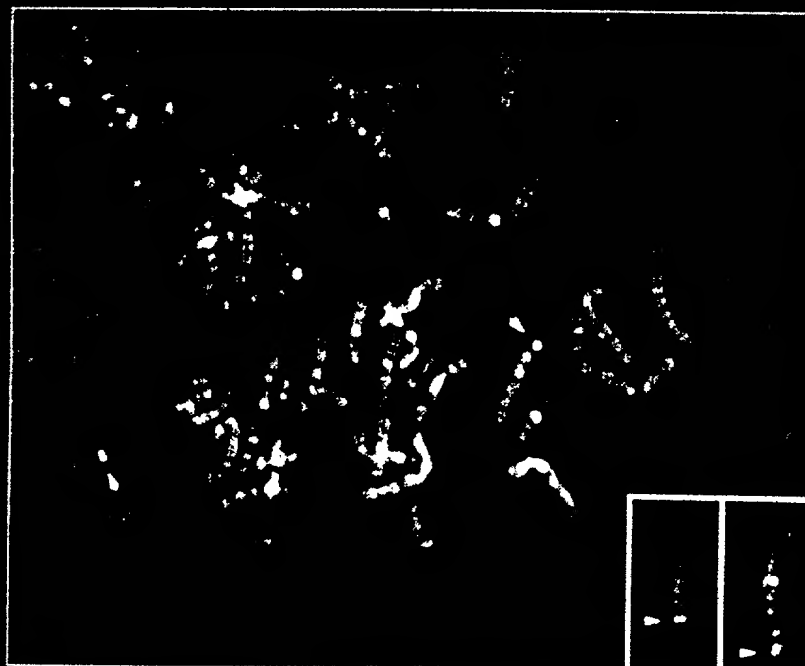


FIGURE 12C

PSCA Maps to Chromosome 8q24.2



Fluorescent
in Situ Hybridization
Analysis of PSCA

FIGURE 13

TOP SECRET 645960

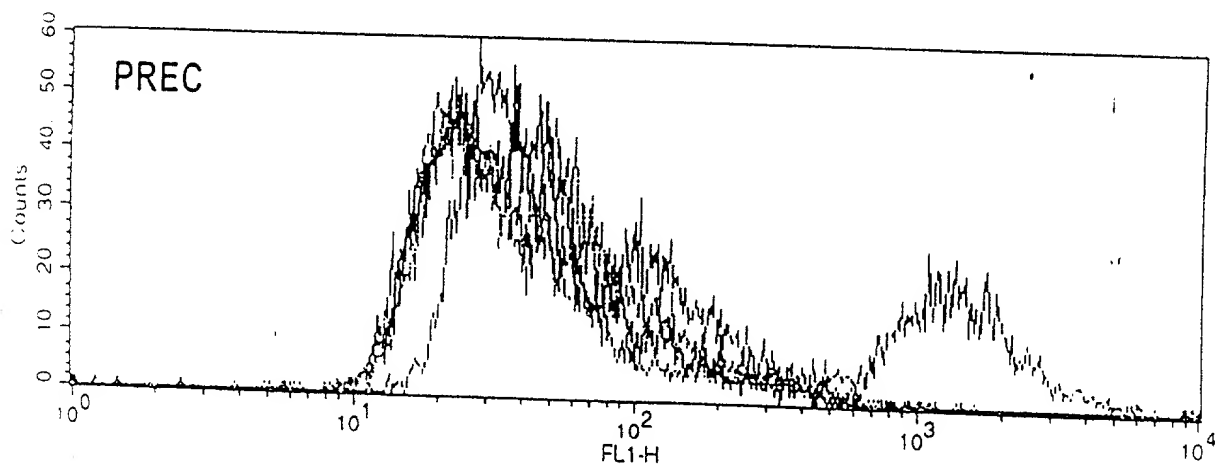
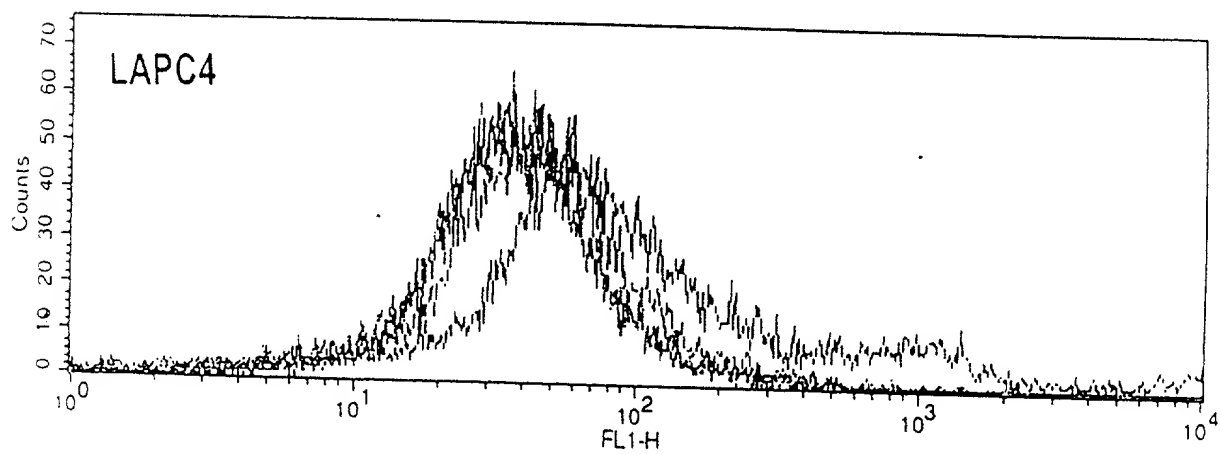
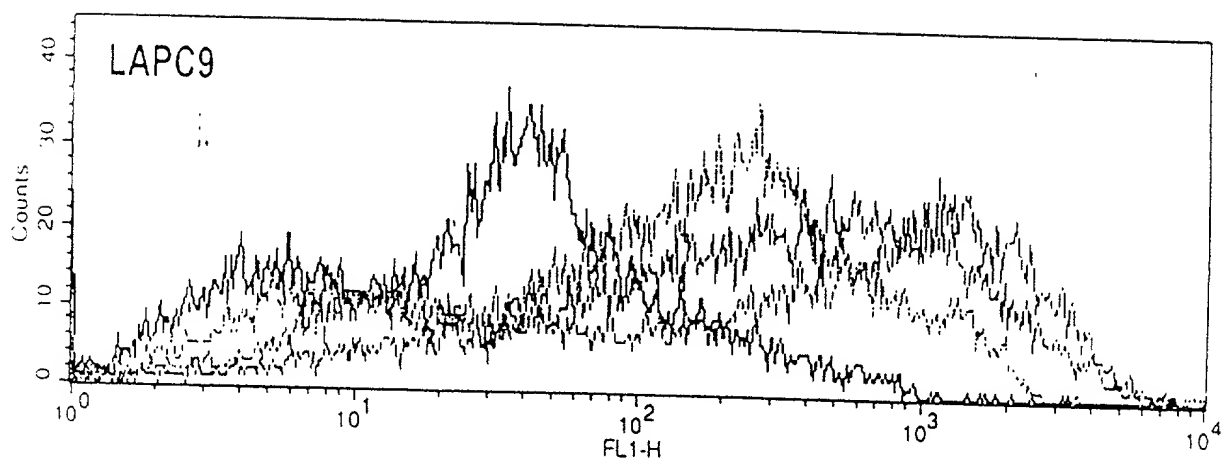


FIGURE 14

A

Epitope map

mAb	Isotype	FL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	lgG1 k	2.039	0.007	0.628	0.000
2H9	lgG1 k	1.318	0.863	0.032	0.021
3C5	lgG2a k	2.893	1.965	0.016	0.005
3E6	lgG3 k	0.328	0.024	0.069	0.370
4A10	lgG2a k	2.039	1.315	0.000	0.014
2A2	lgG2a k	1.366	0.733	0.010	0.003
3G3	lgG2a k	2.805	1.731	0.004	0.000

B

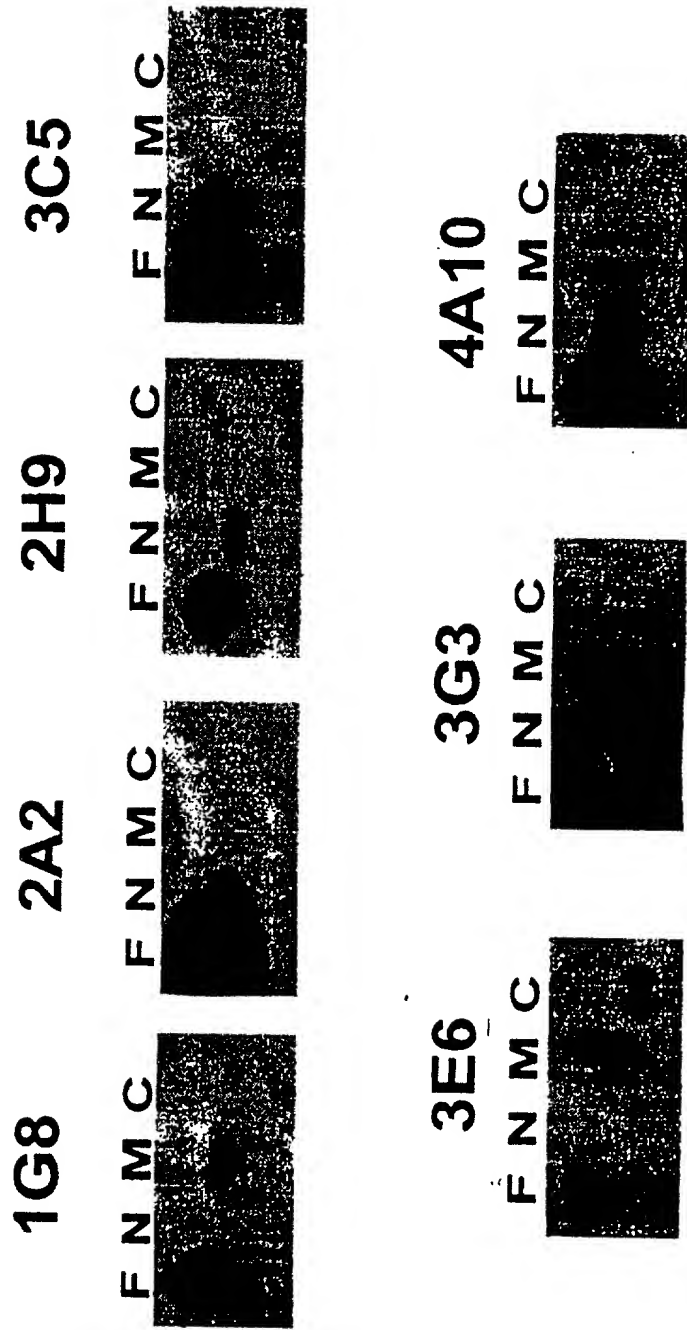


FIGURE 15

[illegible]

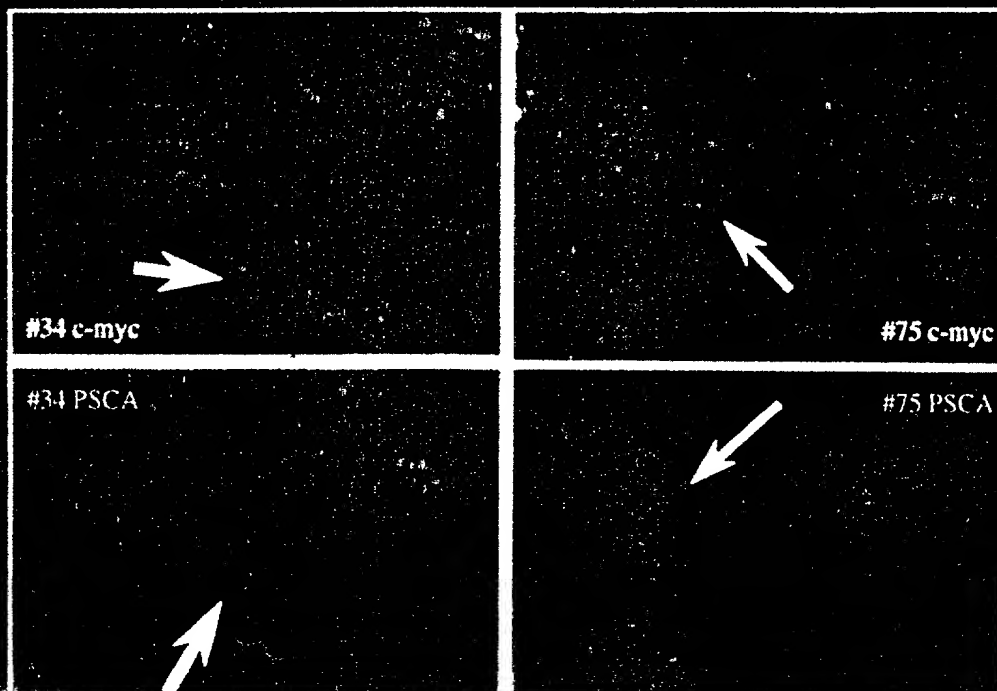
(Reiter, R.E., et al., 1997. *PNAS*)



FISH Analysis of PSCA and c-myc in Prostate Cancer

Gain Chromosome 8

Amplification



R. Jenkins

FIGURE 17

09854139 054401

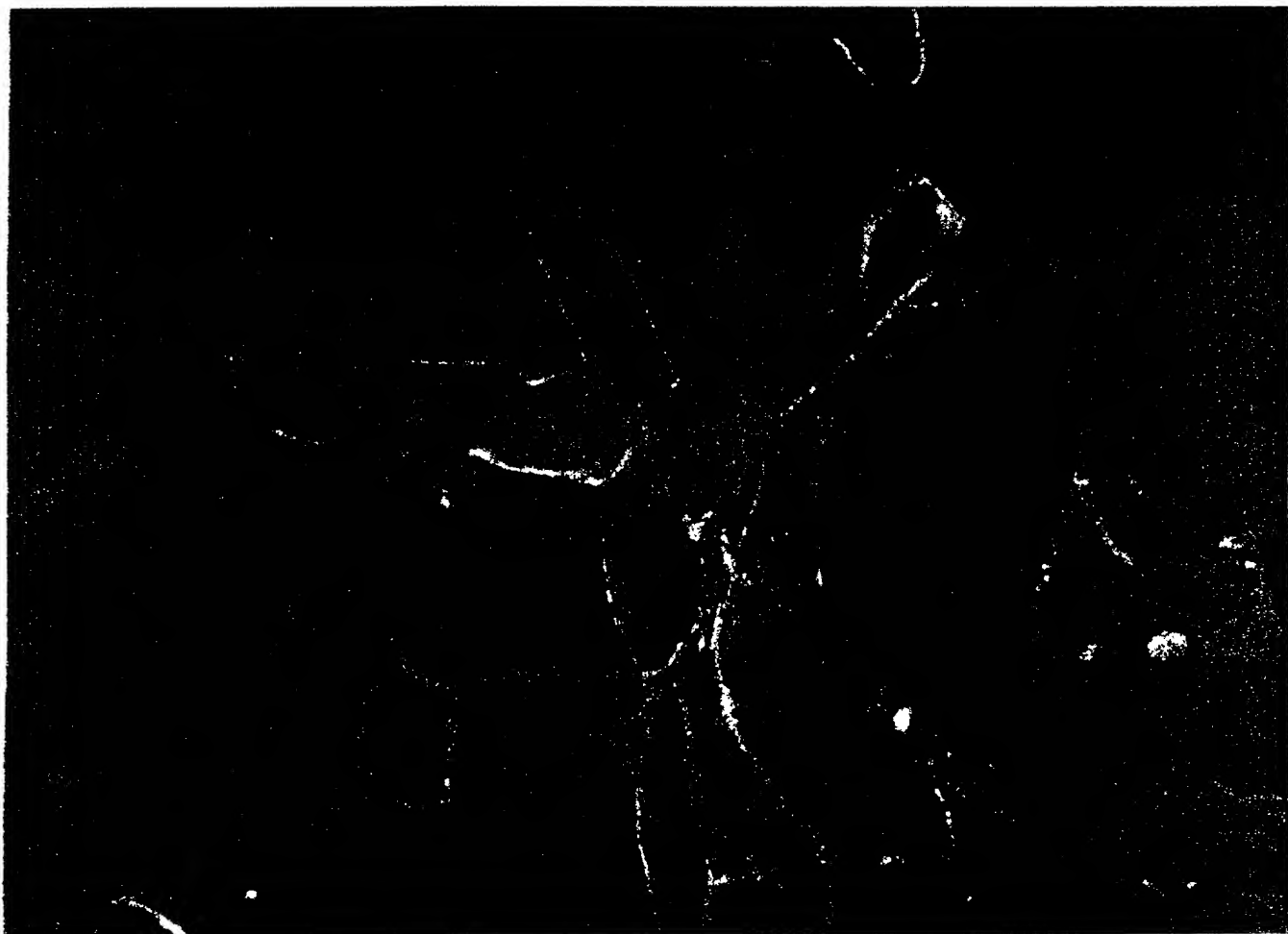


FIGURE 18

TOP SECRET

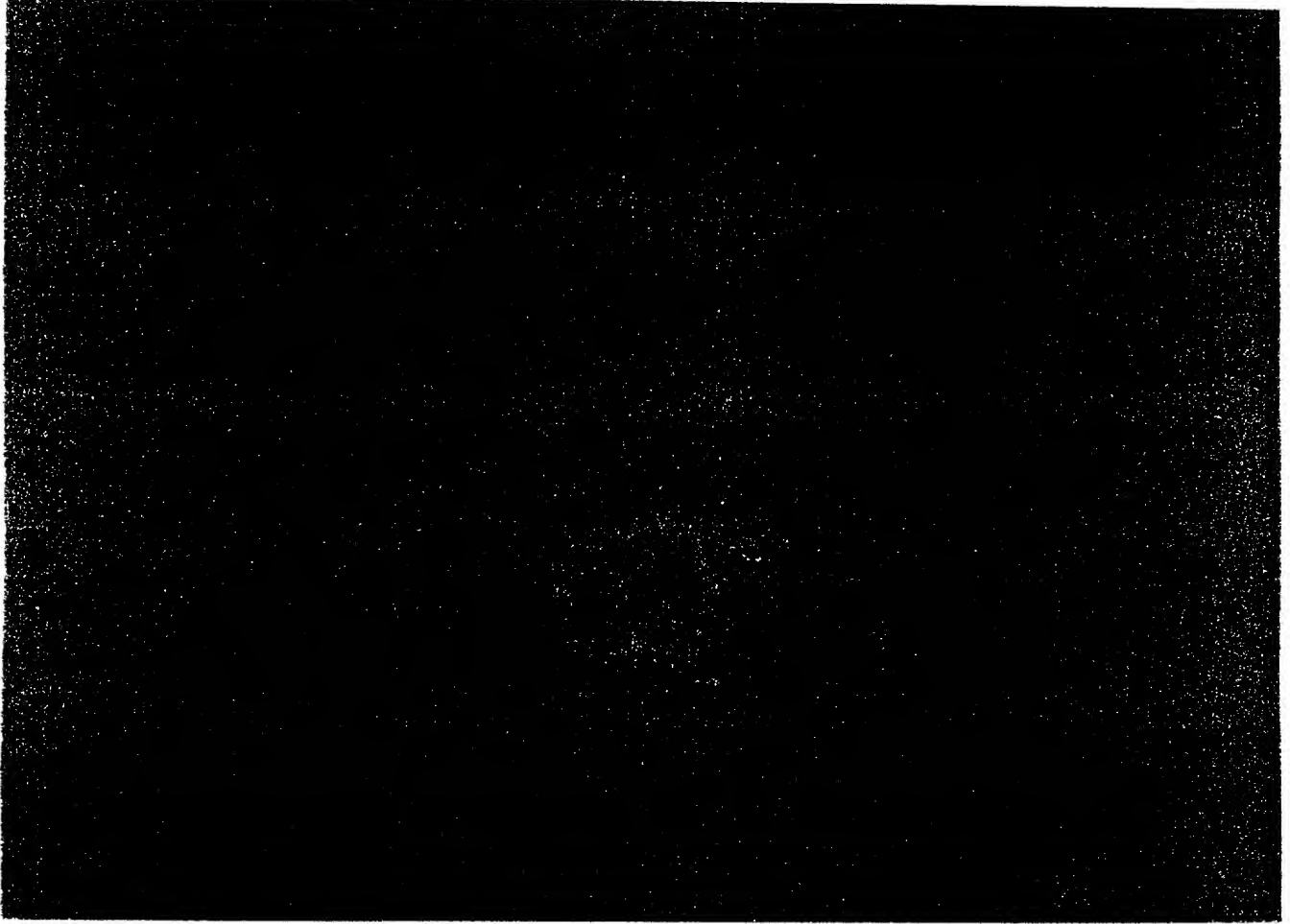


FIGURE 19

FIGURE 20

PSCA Immunostaining of Primary Tumors

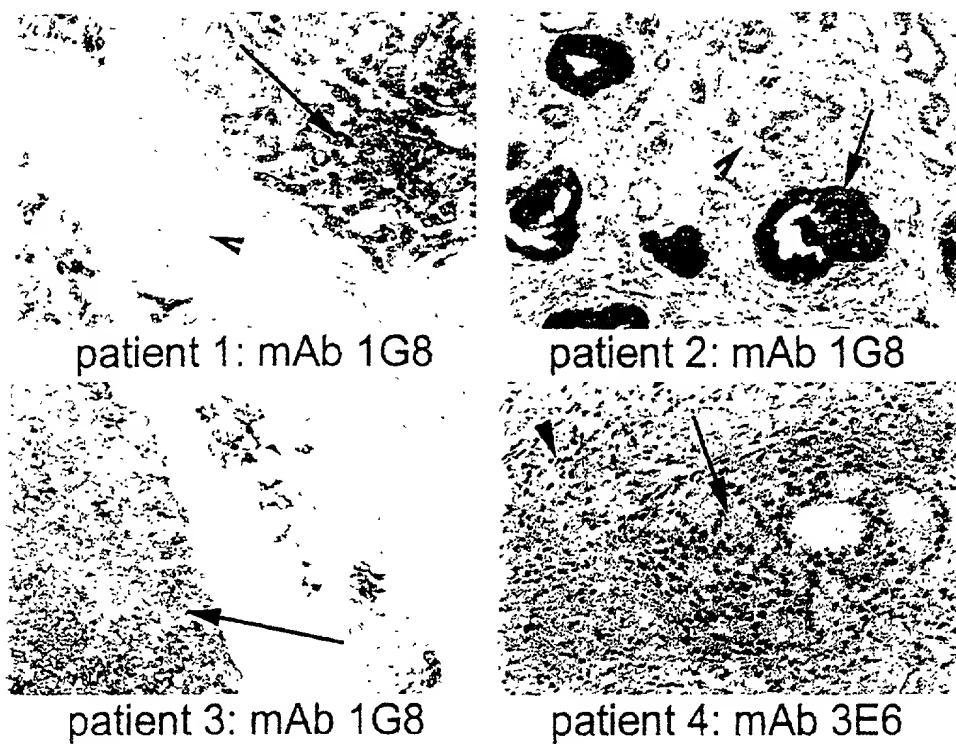


FIGURE 21

FOOTED "ESTES"



FIGURE 22

FOOTNOTES ESTS660



FIGURE 23

This is a high-contrast, black and white micrograph of a histological section. The image displays several large, dark, irregularly shaped structures, likely glandular or ductal in nature, which are surrounded by a lighter, more granular matrix. The structures vary in size and shape, with some showing internal detail. The overall texture is highly detailed and complex, typical of a microscopic view of tissue.

FIGURE 24

0955453-051401

From PSCA
to [unclear] [unclear] [unclear]

FIGURE 25

T. 047510" 6. 51. 53. 50

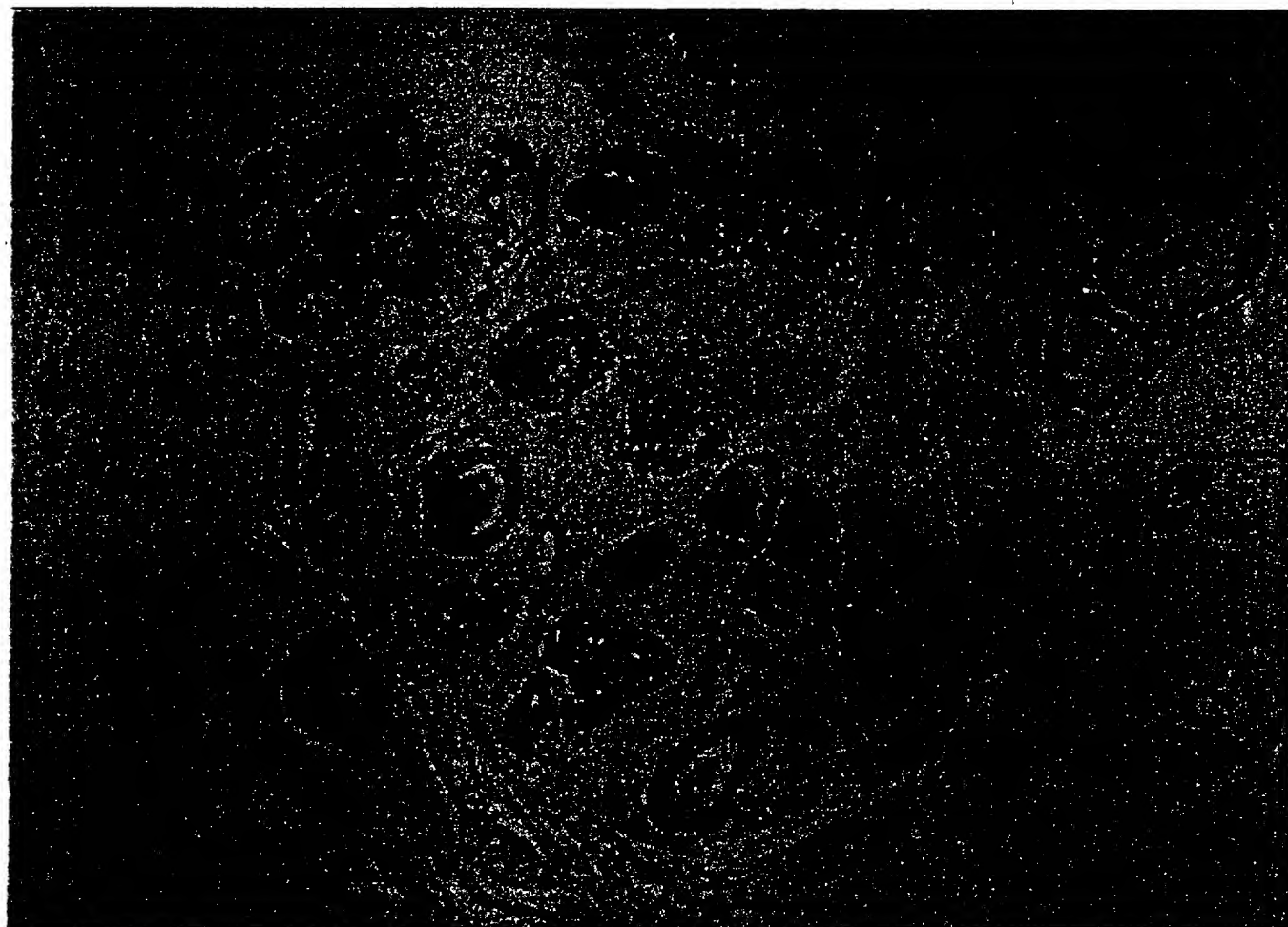
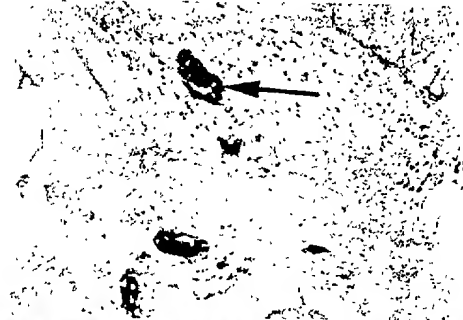


FIGURE 26

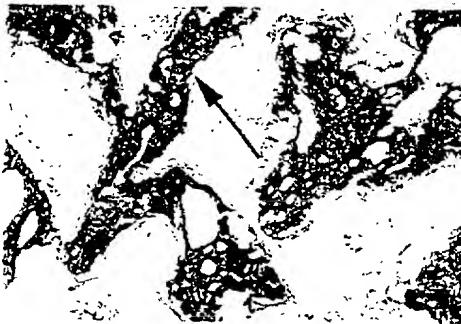
FIGURE 27

Photo EST 5363

PSCA Immunostaining of Bony Metastases



Patient 5: H and E
and mAb 1G8



Patient 4: H and E
and mAb 3E6

FIGURE 28

0985153.054401
"EST 50" EST 50"

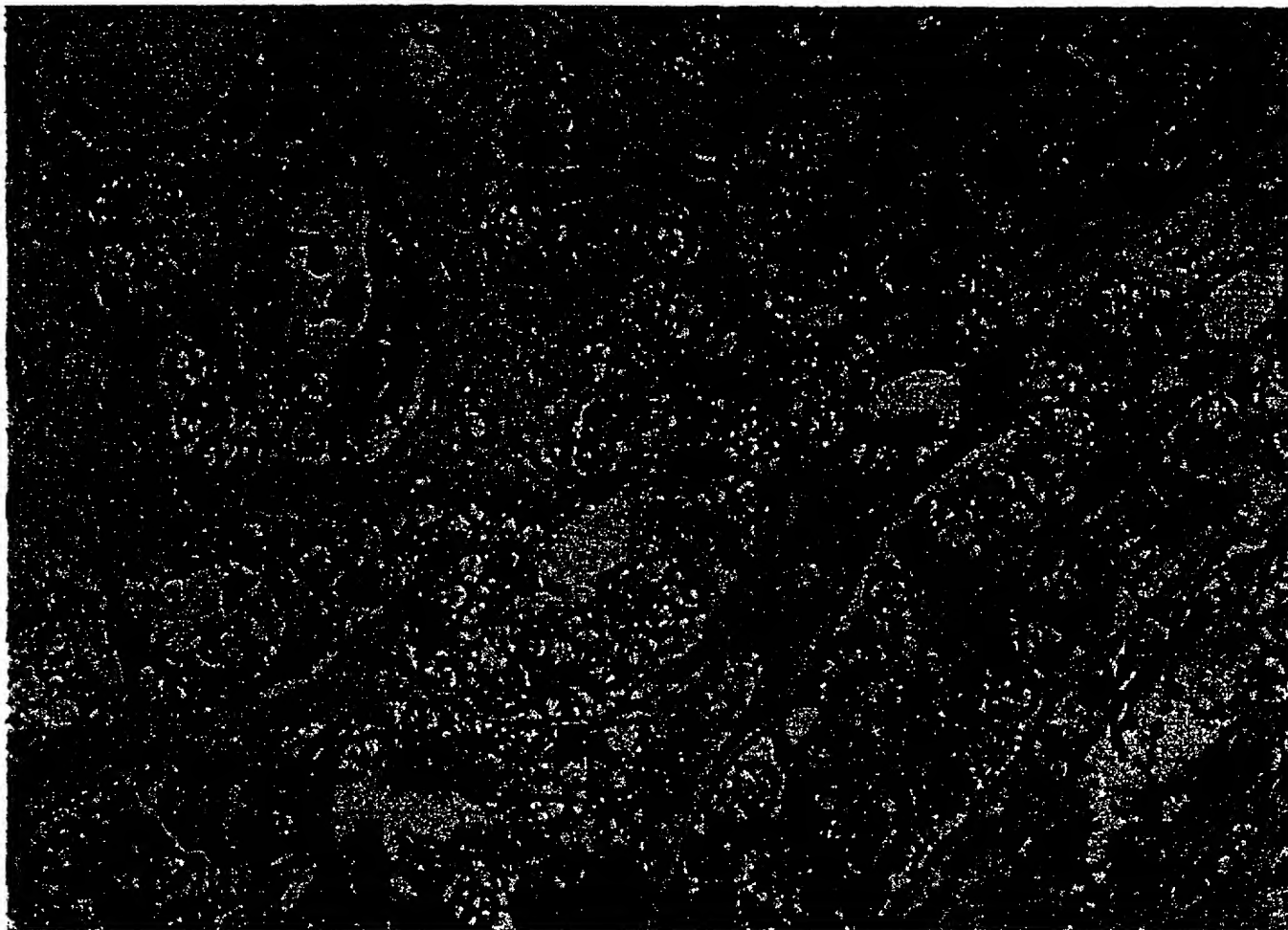


FIGURE 29

A high-contrast, black and white photograph showing a dense, textured surface, possibly a rock face or a wall. The image is characterized by a multitude of small, light-colored spots and streaks scattered across a dark, grainy background, creating a complex, almost abstract pattern. The lighting is harsh, emphasizing the irregularities and textures of the surface.

FIGURE 30

09855153 051401

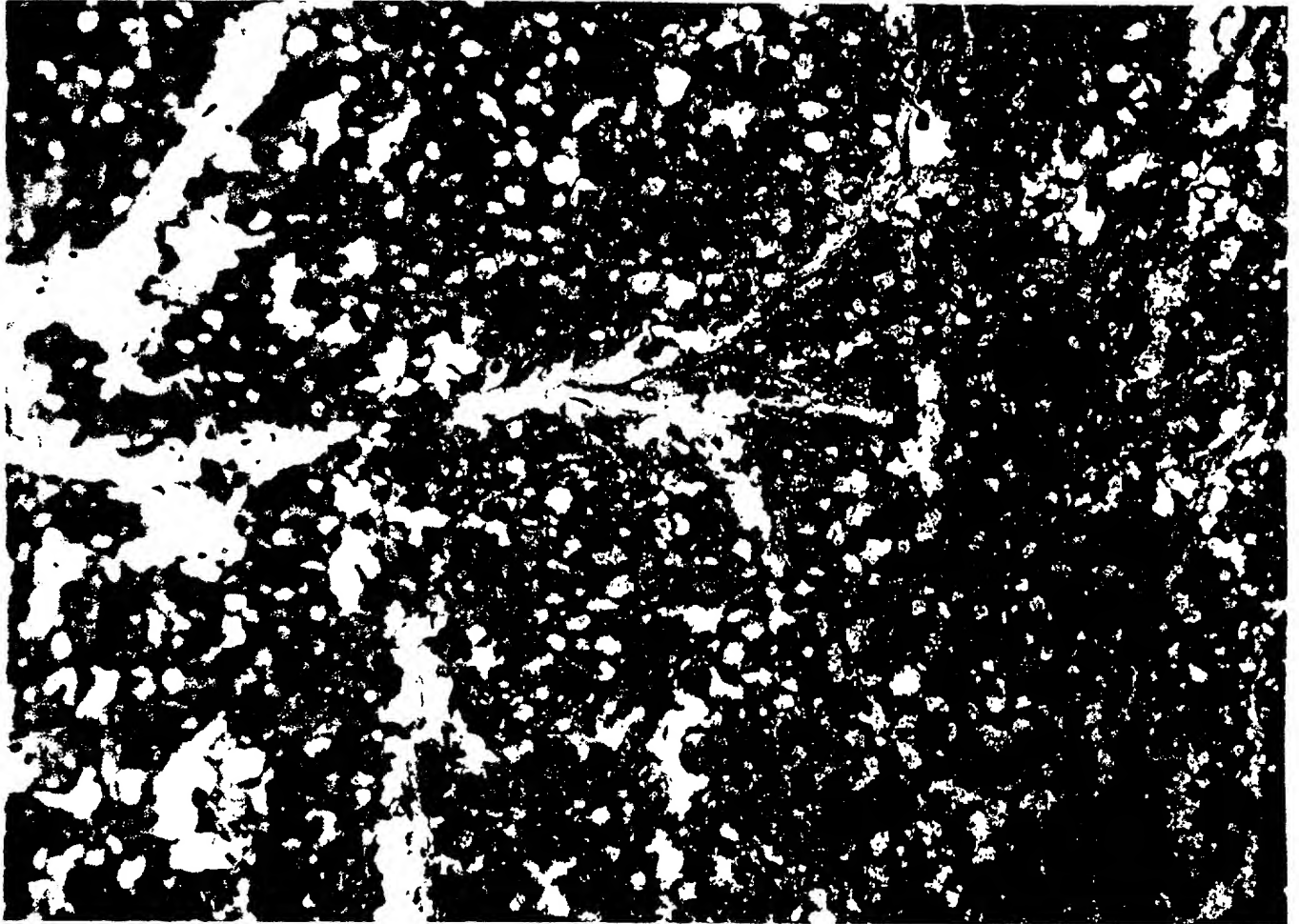


FIGURE 31

0985133-061401

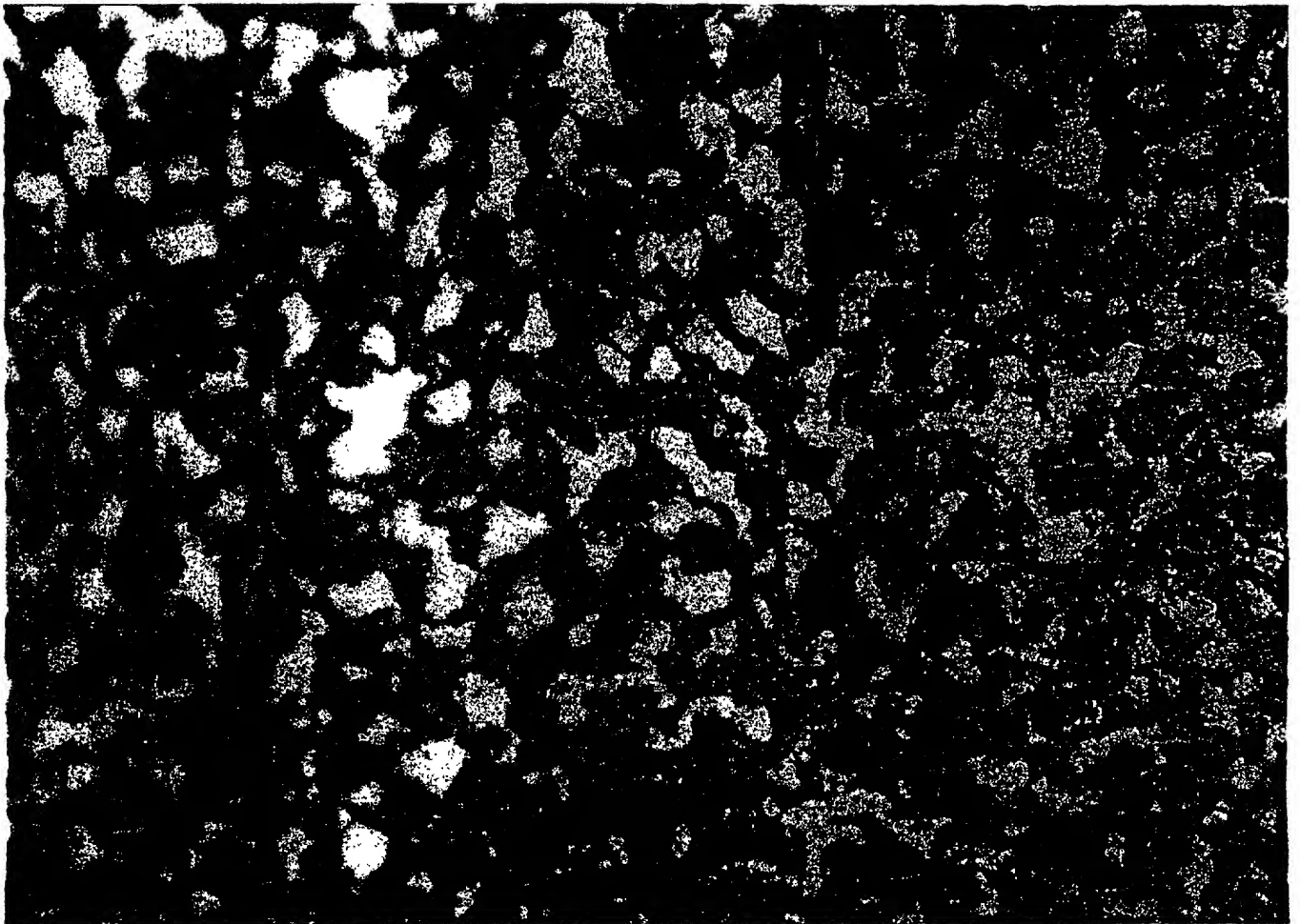


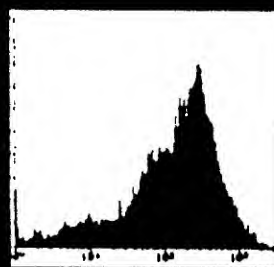
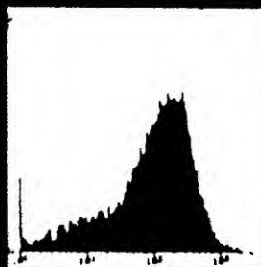
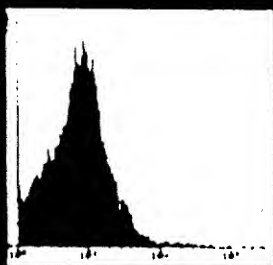
FIGURE 32

PSCA Expression in LAPC-9 Xenograft by FACS

Secondary Antibody

1G8

2H9



4A10

3C5

3E6

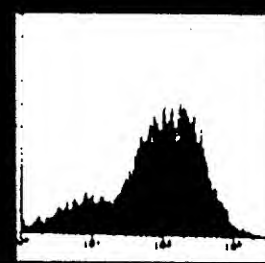
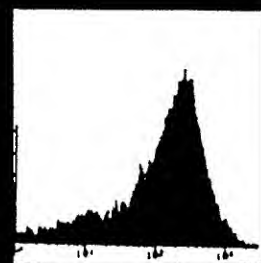
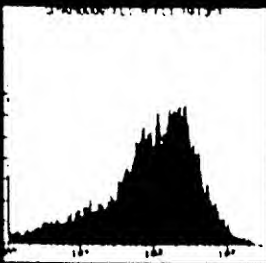


FIGURE 33

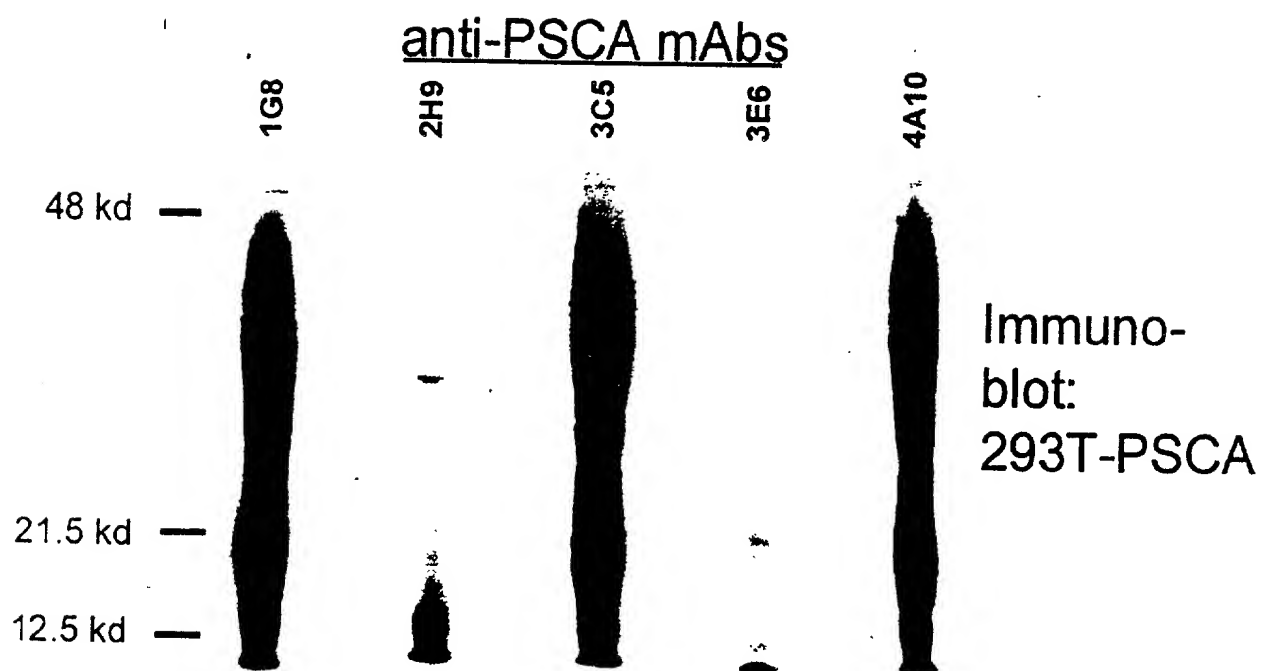


FIGURE 34

Immunofluorescent Staining of LNCaP-PSCA Cells

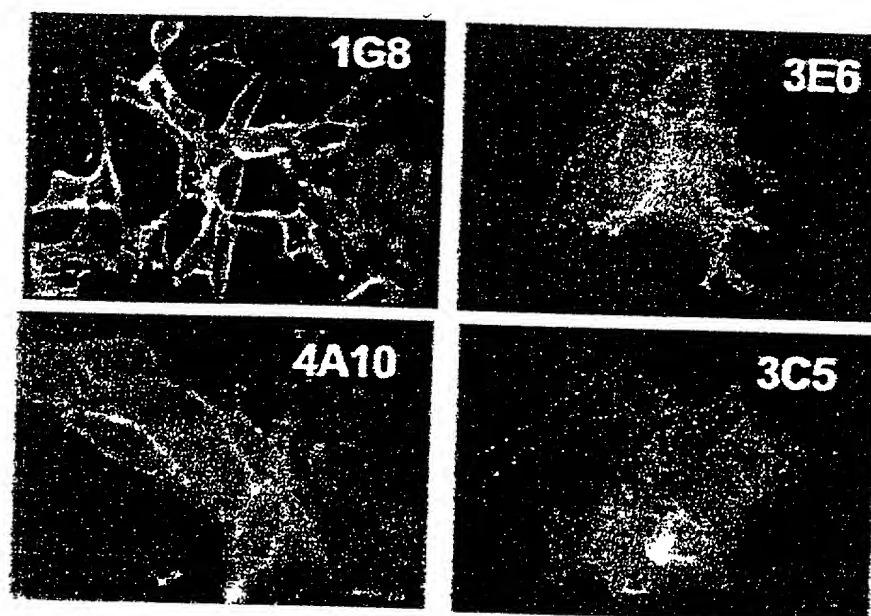


FIGURE 35

TOP SECRET

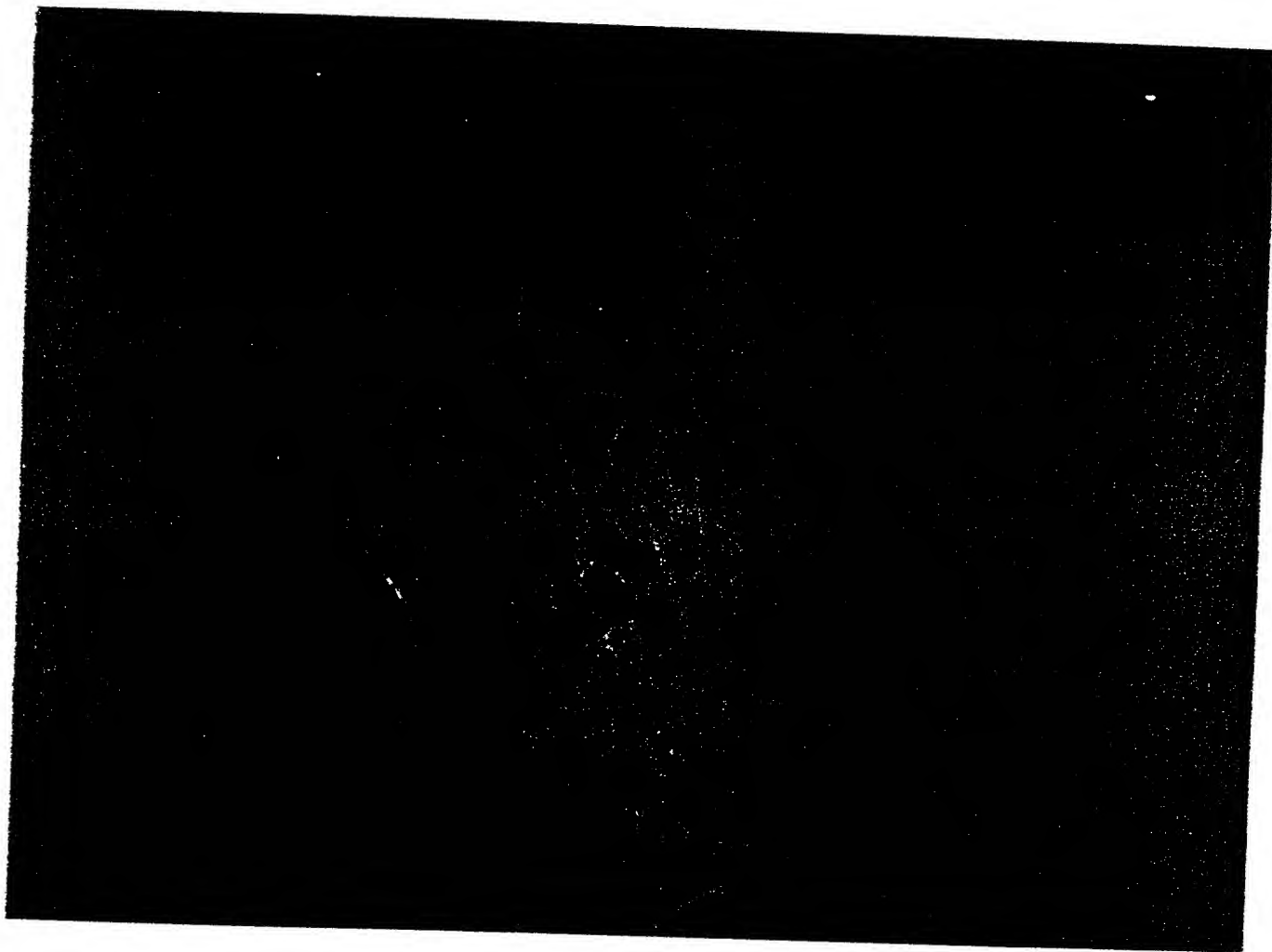


FIGURE 36

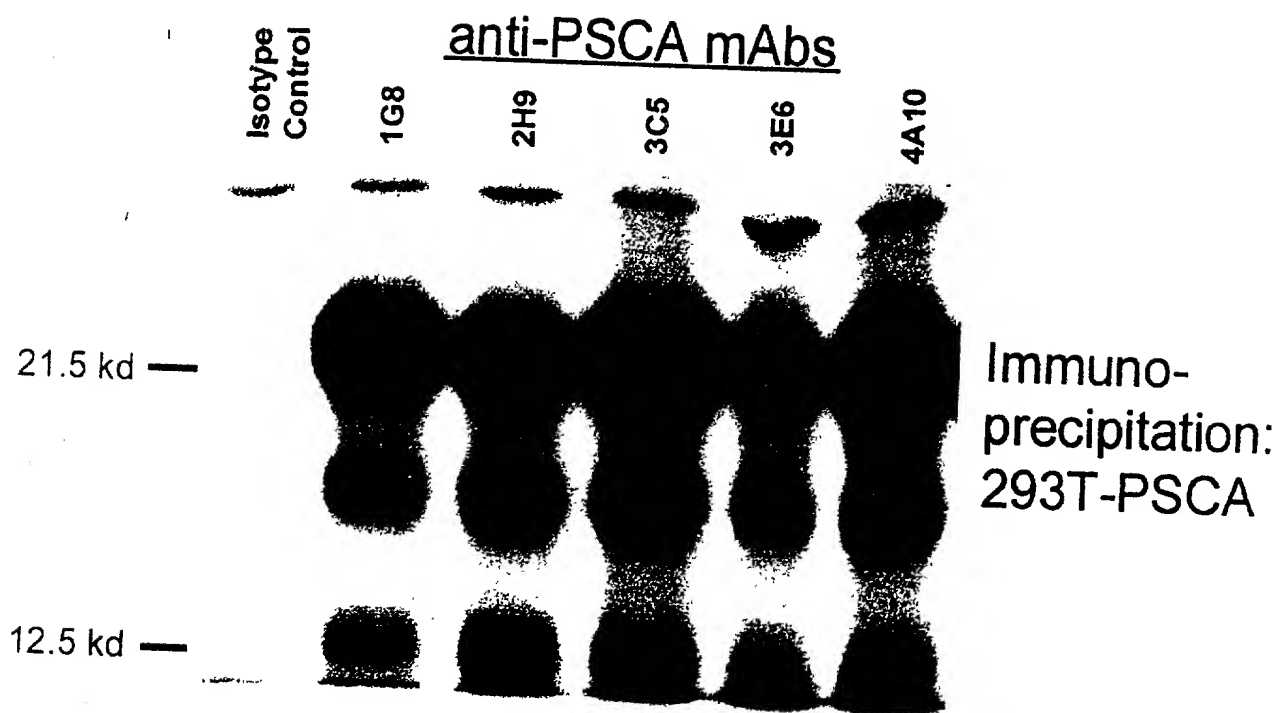


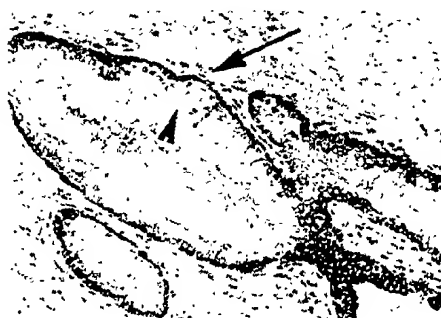
FIGURE 37

Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8

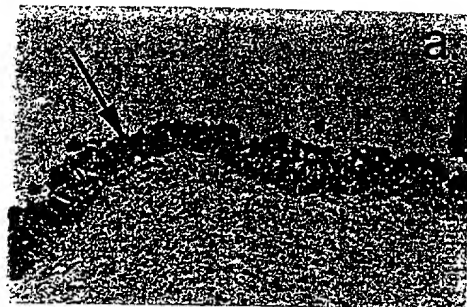


Atrophy: PSCA mAb 2H9

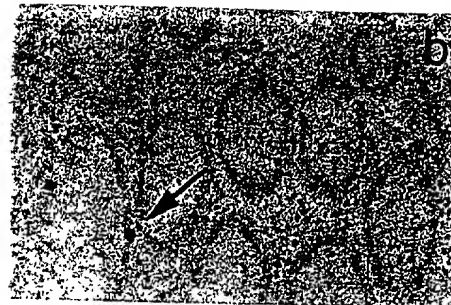


FIGURE 38

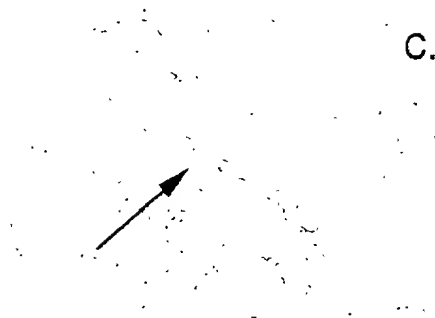
A.



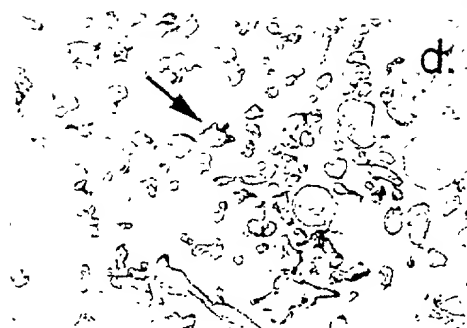
Bladder: 1G8



Colon: 1G8



Kidney: 3E6



Placenta: 3E6

B.

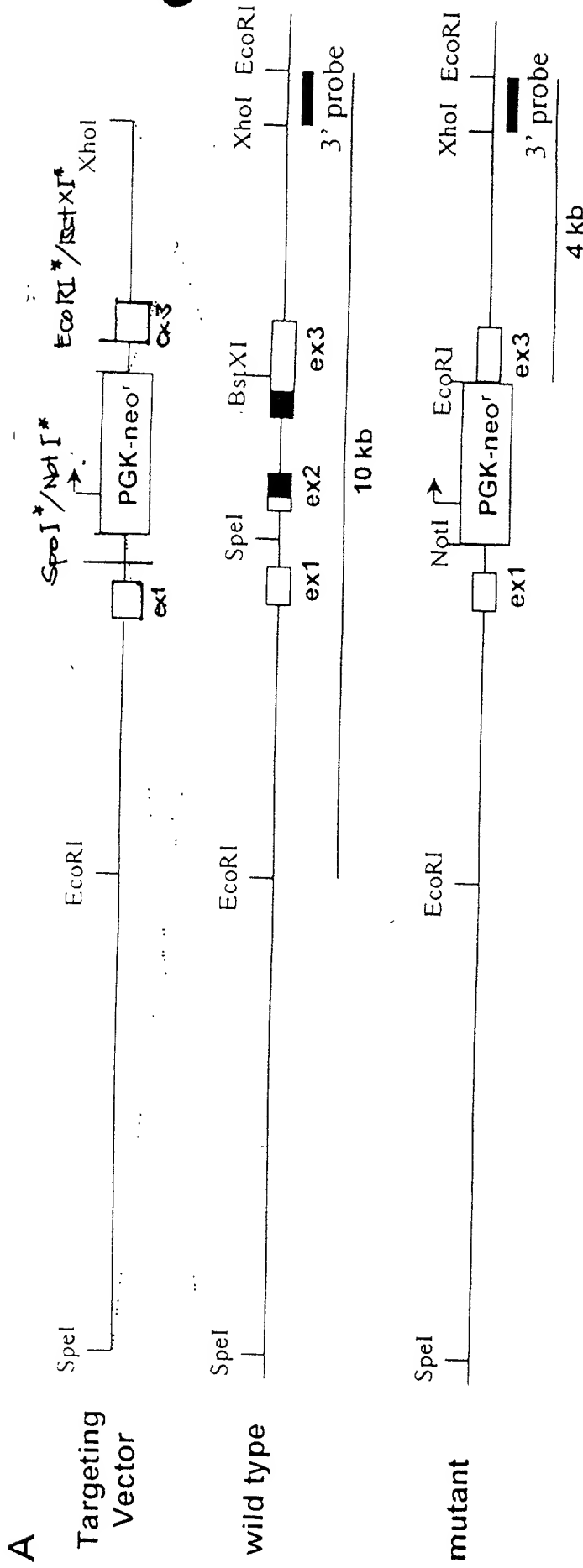


PSCA

Actin

FIGURE 39

Targeting of Mouse PSCA Gene



B. Genomic Southern Analysis of ES Cells

- * ex1, 2, and 3 are the exons of PSCA gene.
- * Black boxes of ex2 and ex3 encode PSCA mature protein sequences.
- * ES genomic DNAs were digested with EcoRI, followed by Southern hybridization using 3' probe.

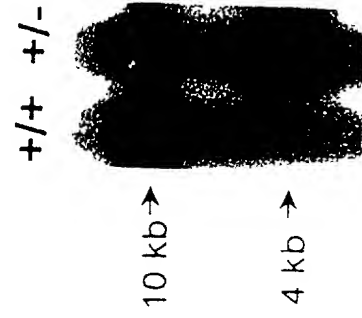
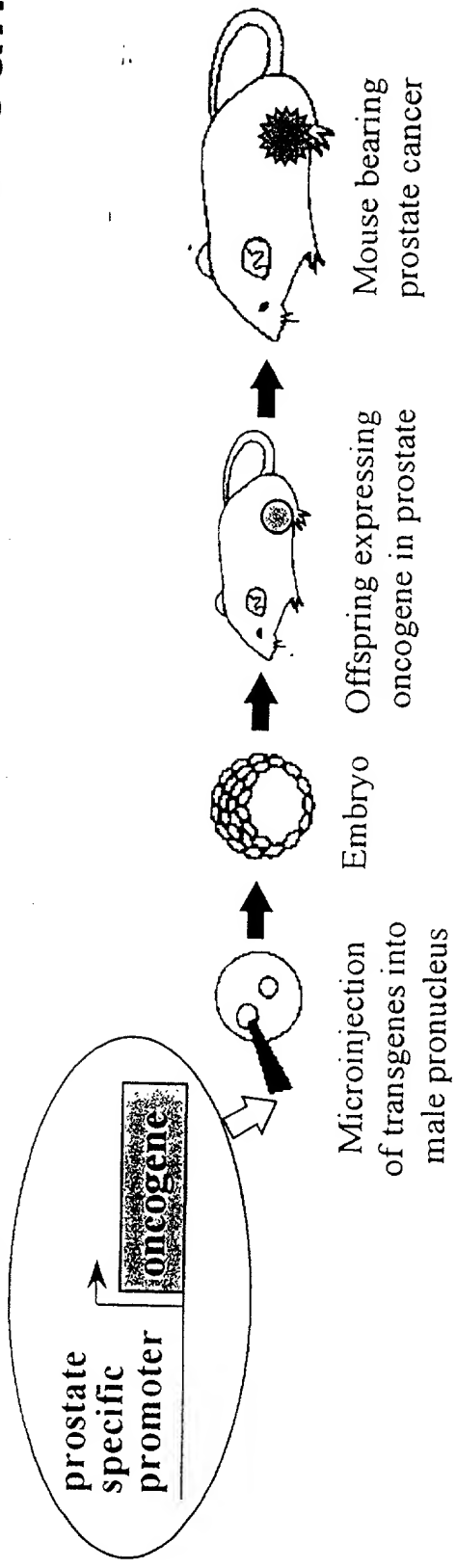


FIGURE 40

Transgenic Mouse Models of Prostate Cancer



Transgene	Target tissues	Characteristics
C3(1) (-3 kb)/ SV40 large+small T <i>Maroulakou et al.</i> 1994 <i>PNAS</i>	prostate (secretory cells) urethral, mammary and sweat gland	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 28 wks No metastases
Probasin (-426 bp)/ SV40 large+small T <i>Greenberg et al.</i> 1995 <i>PNAS</i>	prostate (secretory cells)	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcinoma 12 wks Metastases in lymph node, lung, liver and bone
Cryptdin2 (-6.5 kb)/ SV40 large+small T <i>Garabedian et al.</i> 1998 <i>PNAS</i>	prostate (neuroendocrine cells) small intestine	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 16 wks Metastases in lymph node, lung, liver and bone

FIGURE 41

Reporter Gene Constructs for Transfection Assay

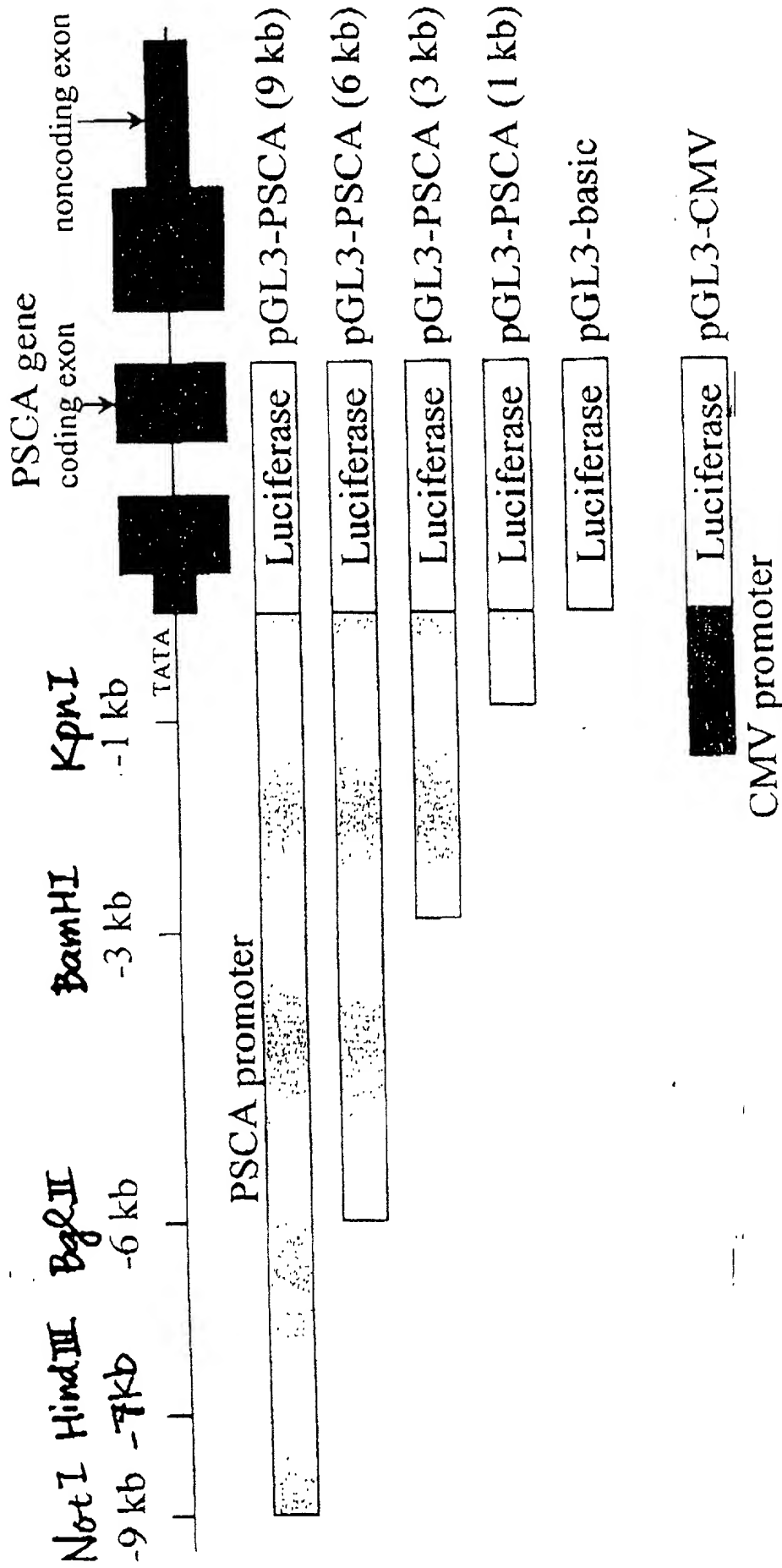


FIGURE 42

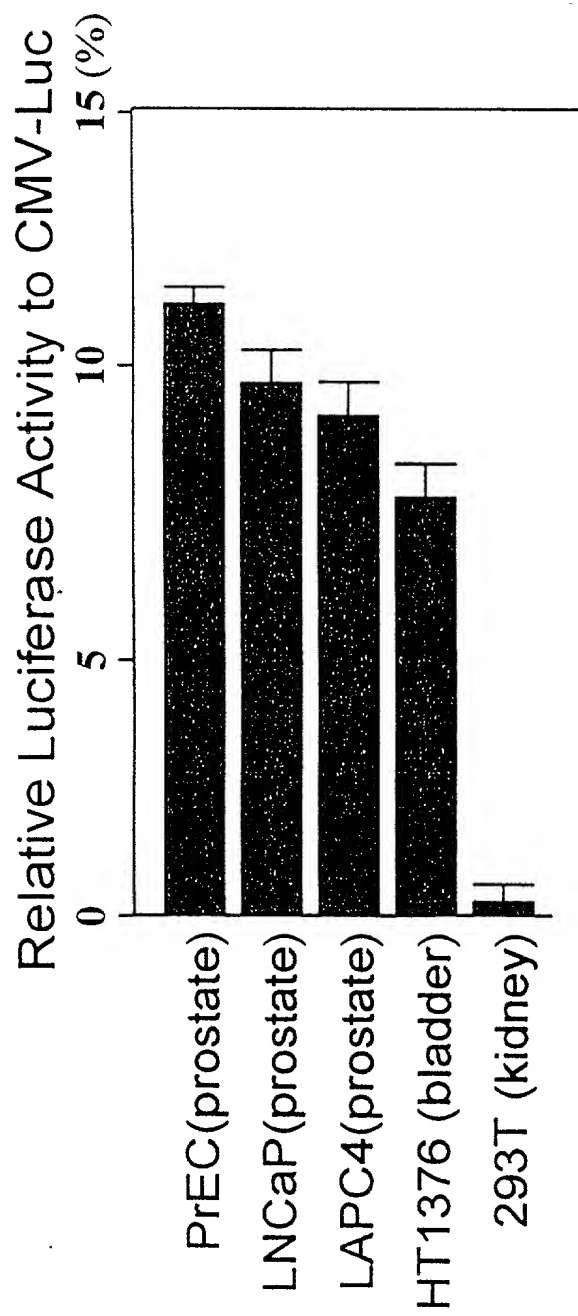


FIGURE 43

Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

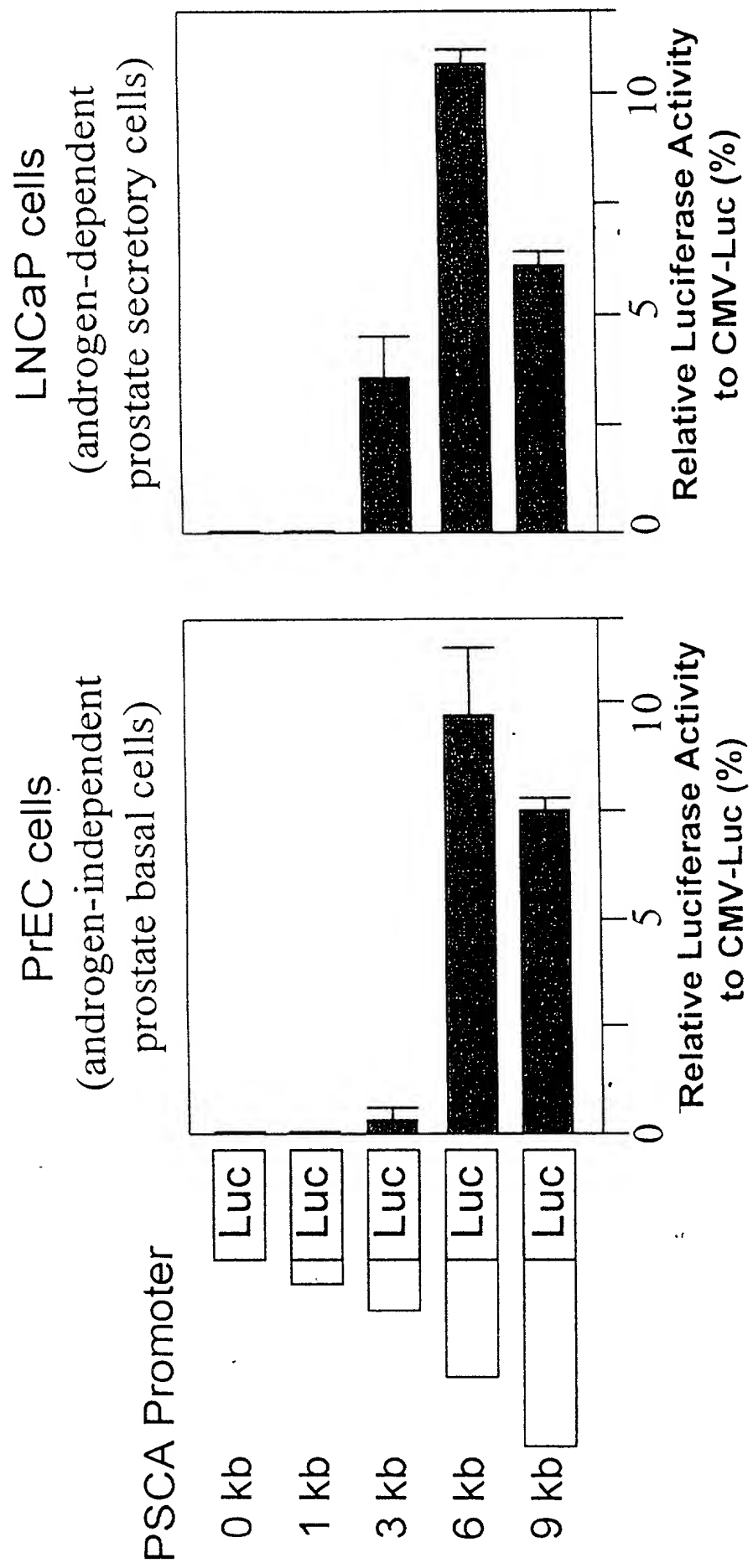


FIGURE 44

Update of Transgenic Mouse Projects

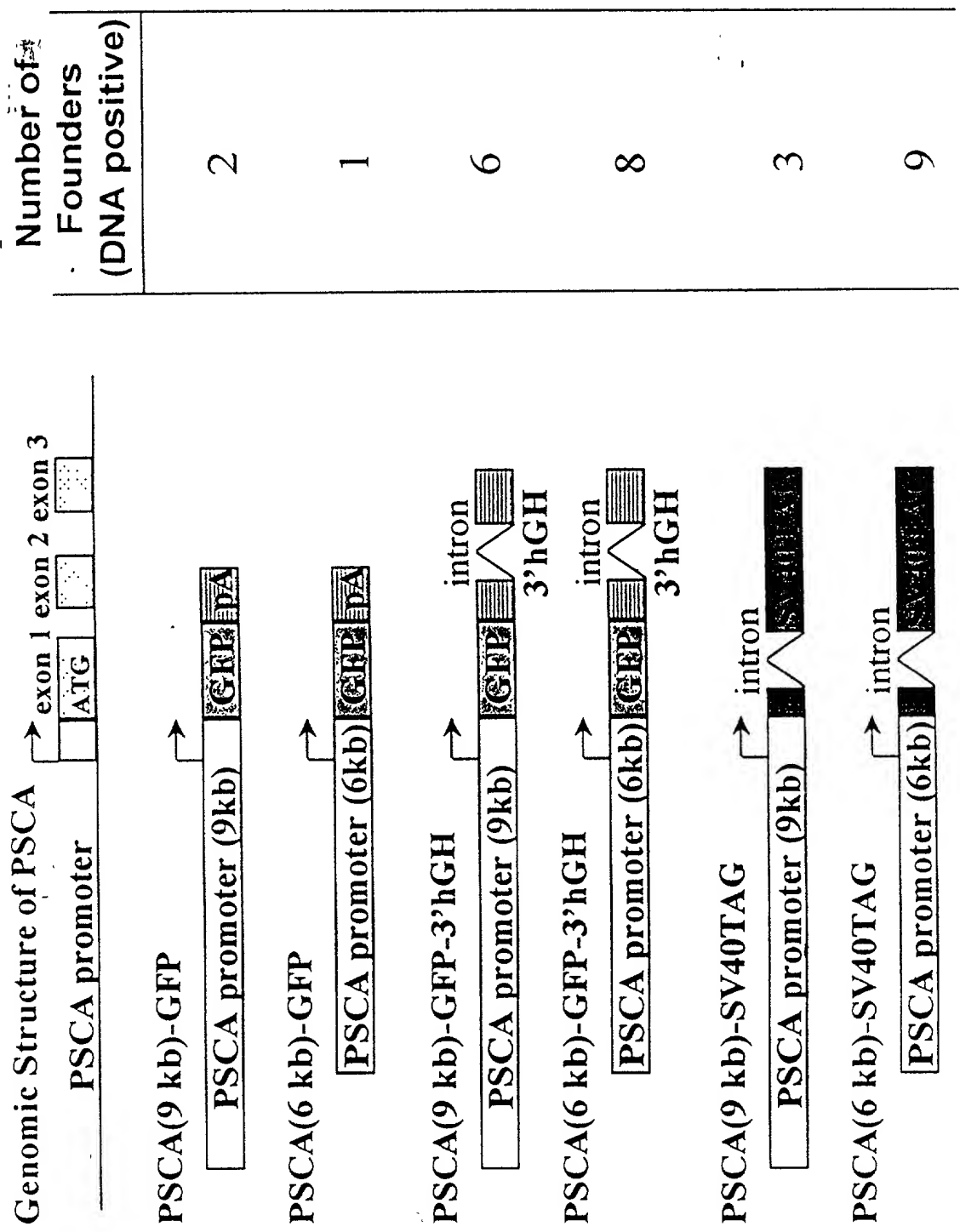
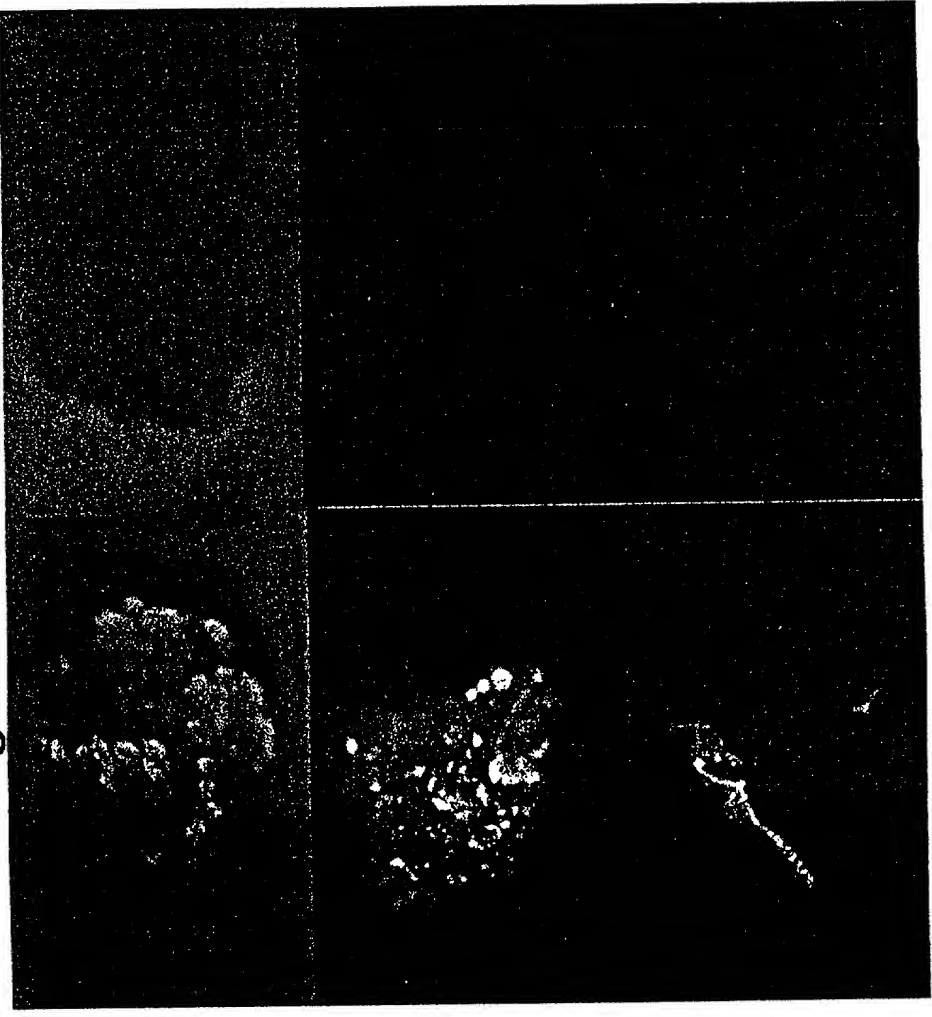


FIGURE 45

Negative tissues

Stomach
 Small intestine
 Colon
 Seminal Vesicle
 Urethra
 Testis
 Liver
 Kidney
 Lung
 Brain
 Heart
 Skeletal muscle
 Ovary
 Uterus

Whole-mount green fluorescence image
 Transgenic Non-transgenic



Prostate
 (A25-106-2)

Bladder
 (A25-104)

Skin
 (A25-106-2)

HUMAN

Spleen
Thymus
Prostate
Testis
Ovary
S. int.
Colon
PBL

Heart
Brain
Placenta
Lung
Liver
Muscle
Kidney
Panc.

hPSCA →

Northern Analysis

MOUSE

Ant. prostate
Dorso/Lat. prostate
Ventral prostate
Bladder
Semin. vesicle
Urethra
Testis
Kidney
Esophagus
Cardiac stomach
Body of stomach
Pyloric stomach
Duodenum
Small intestine
Colon
Salivary gland
Spleen
Thymus
Bone marrow
Skeletal muscle
Heart
Brain
Eye
Lung
Liver
Skin

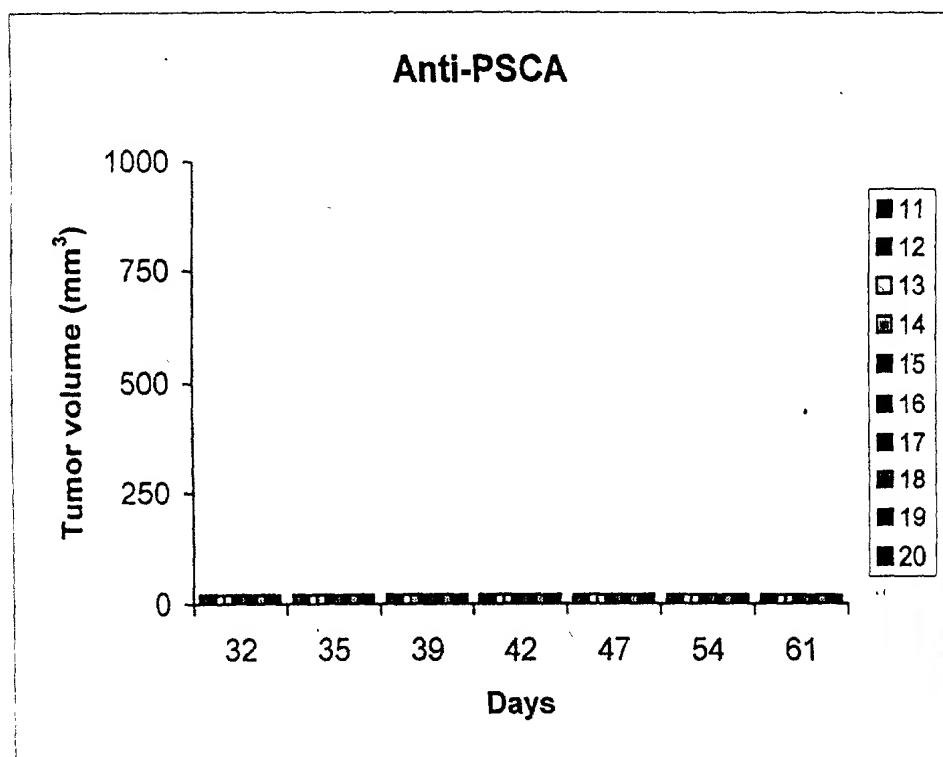
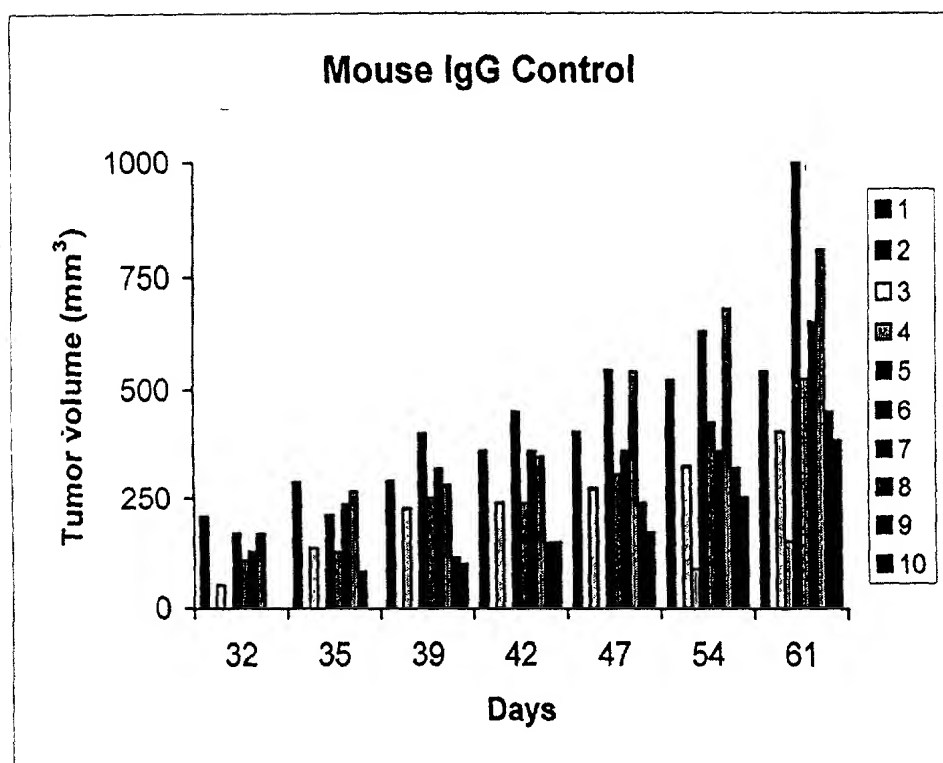
mPSCA →

mG3PDH →

RT-PCR

FIGURE 47

Variable	Mean		SD		t		p	
	Control	Case	Control	Case	Control	Case	Control	Case
Age	30.5	30.5	1.2	1.2	0.0	0.0	0.999	0.999
Gender	15	15	0	0	0.0	0.0	0.999	0.999
Education	12.5	12.5	1.0	1.0	0.0	0.0	0.999	0.999
Occupation	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Marital status	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Religion	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Income	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Family size	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Urban/rural	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Health status	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Smoking status	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Alcohol consumption	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Exercise	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Stress	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Depression	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Anxiety	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Anger	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Loneliness	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Life satisfaction	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Quality of life	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Health-related quality of life	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Physical health	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Mental health	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Social health	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Environmental health	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999
Overall health	1.5	1.5	0.5	0.5	0.0	0.0	0.999	0.999



A

FIG. 49

Epitope recognized (OD 450 nm)

<u>mAb</u>	<u>Isotype</u>	<u>F (18-98)</u>	<u>N (2-50)</u>	<u>M (46-109)</u>	<u>C (85-123)</u>
1G8	IgG1 k	1.485	0.004	1.273	0.003
2A2	IgG2a k	0.973	0.631	0.023	0.010
2H9	IgG1 k	1.069	1.026	0.002	0.001
3C5	IgG2a k	1.916	1.709	0.006	0.002
3E6	IgG3 k	1.609	0.036	1.133	2.118
3G3	IgG2a k	2.805	1.731	0.004	0.000
4A10	IgG2a k	1.053	0.493	0.000	0.001

B

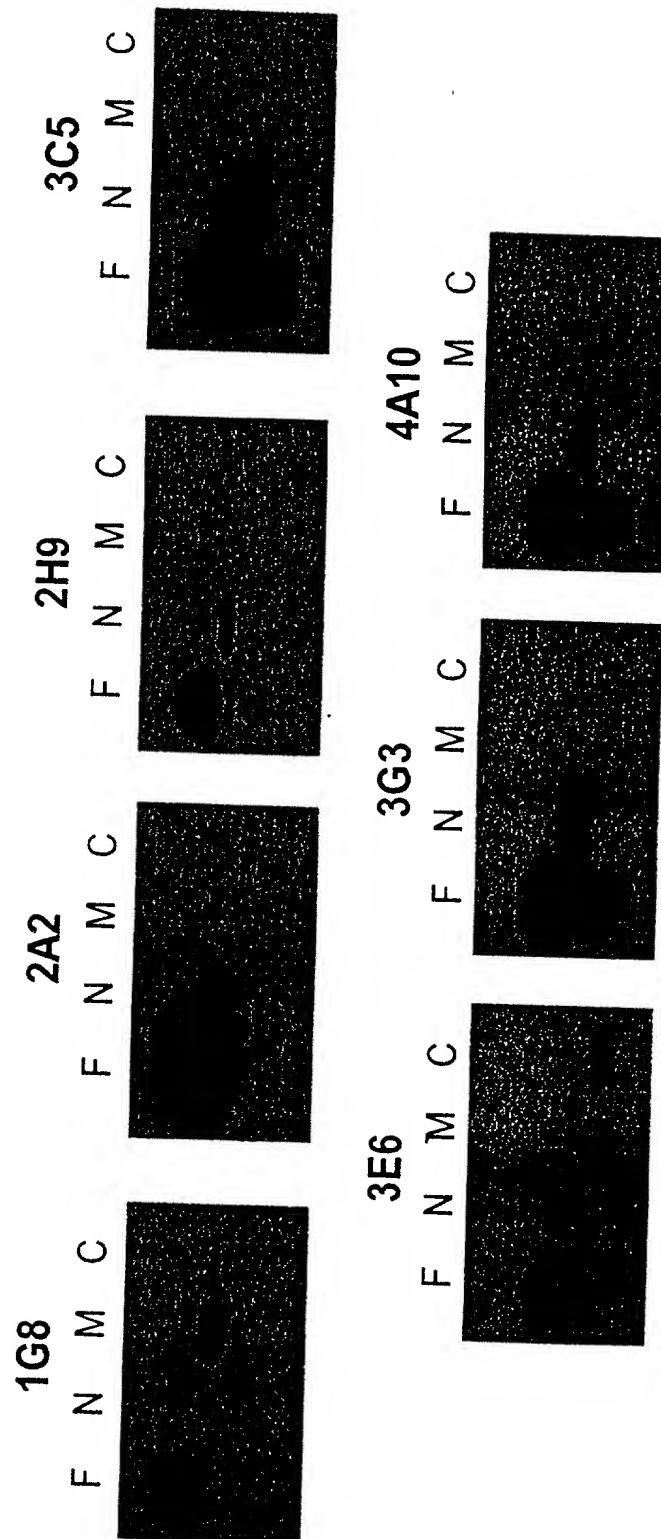
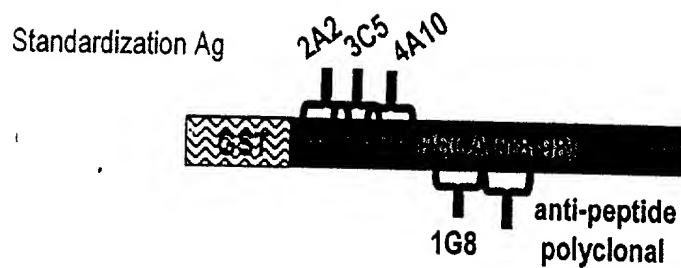
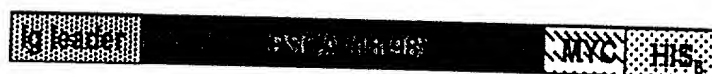


FIG. 50

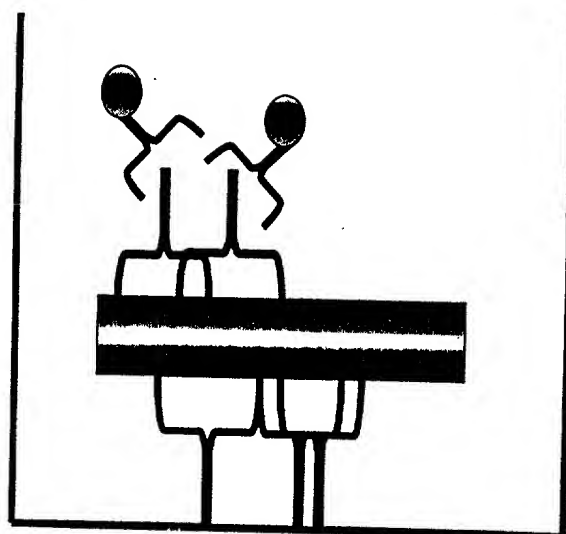
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

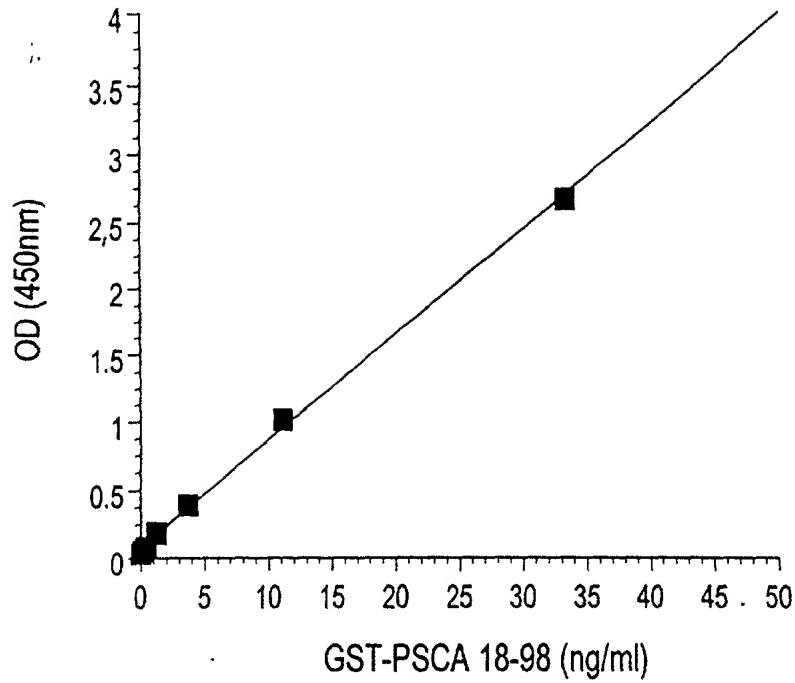
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal
+ mAb 1G8 (IgG1)

FIG. 51

A



B

<u>Sample</u>	<u>OD+range (n=2)</u>	<u>ng/ml</u>
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

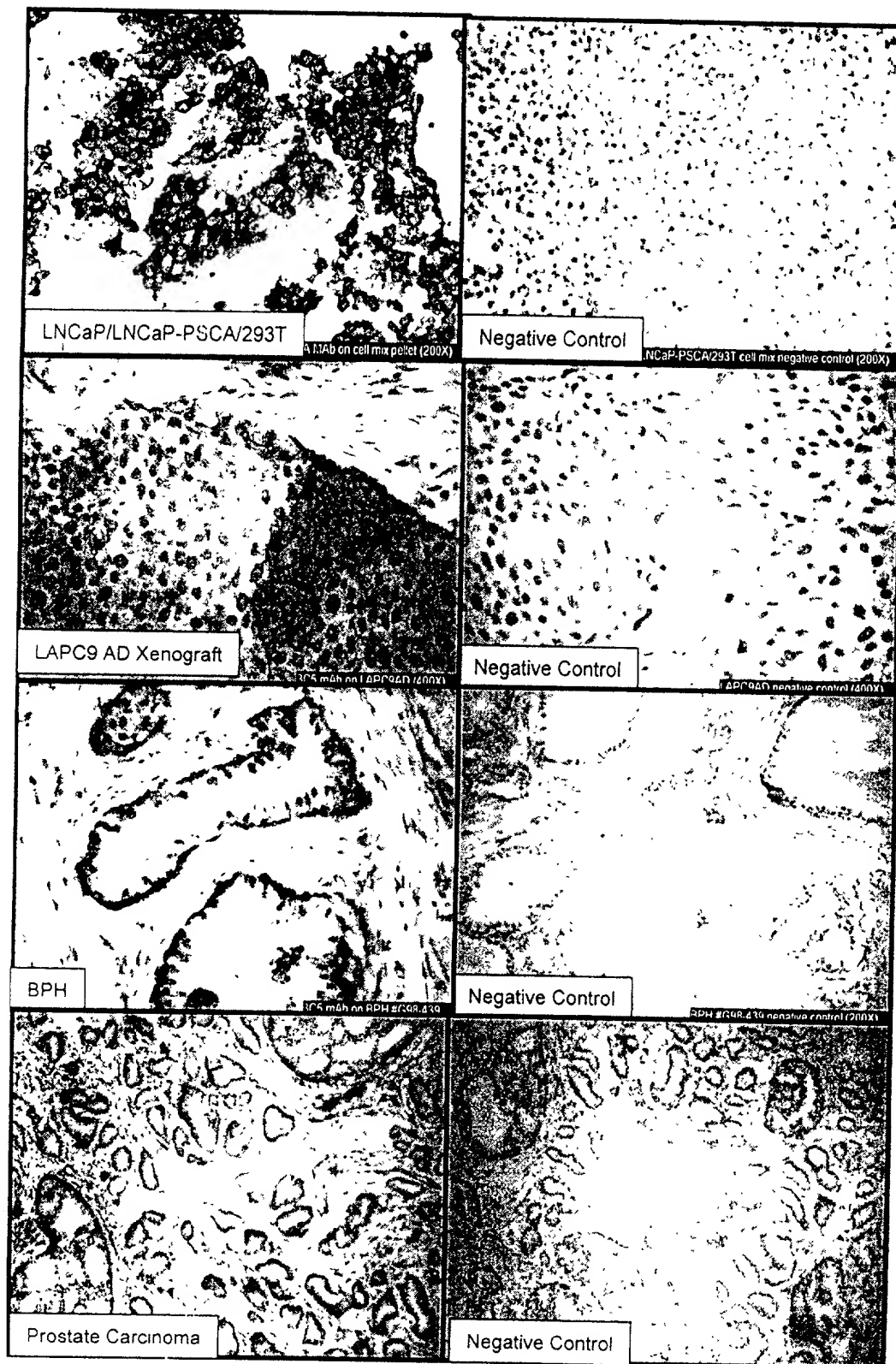


FIG. 52

FIG. 53

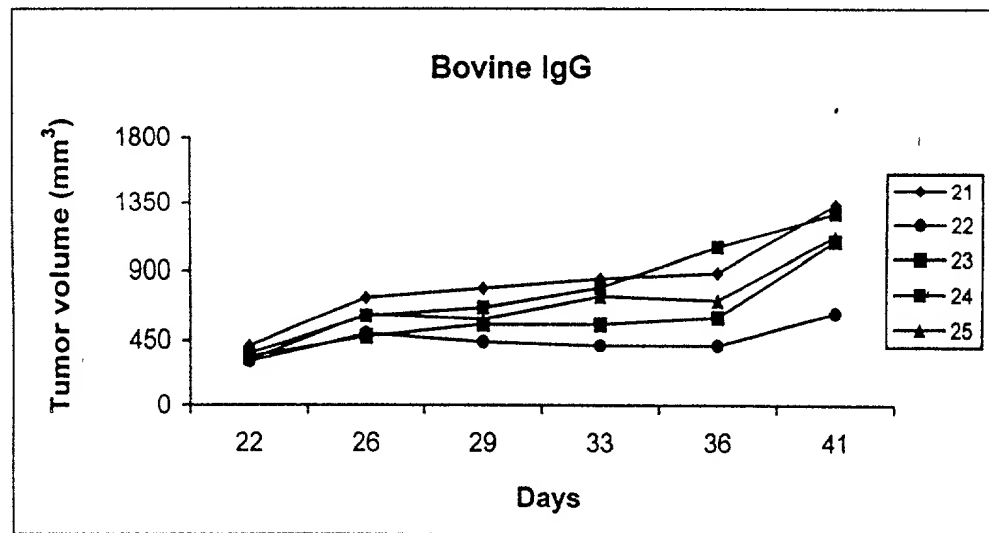
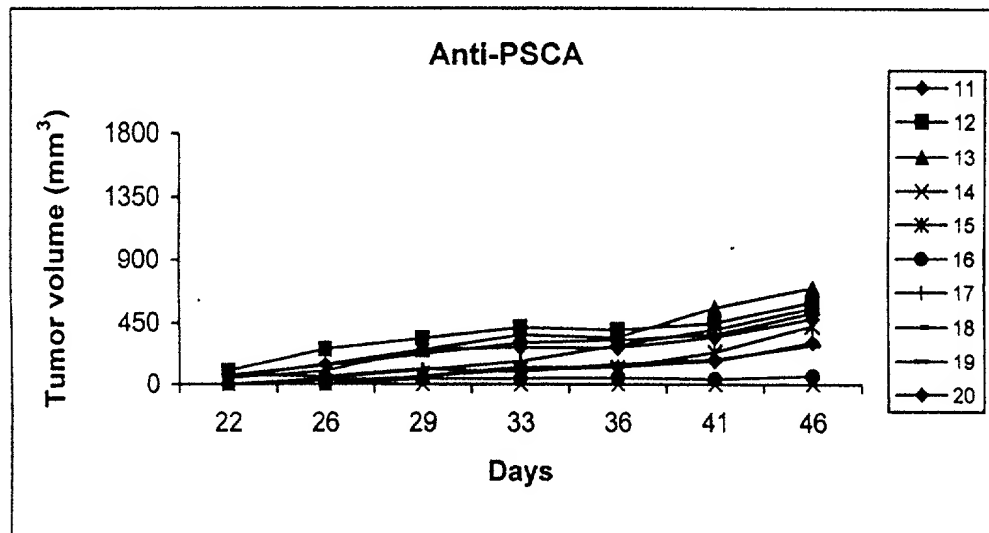
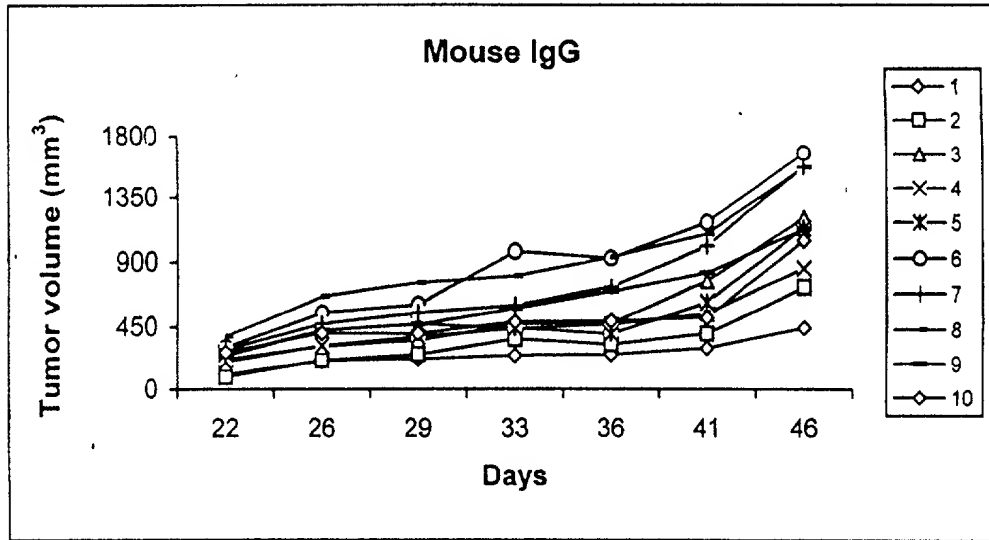


FIG. 54

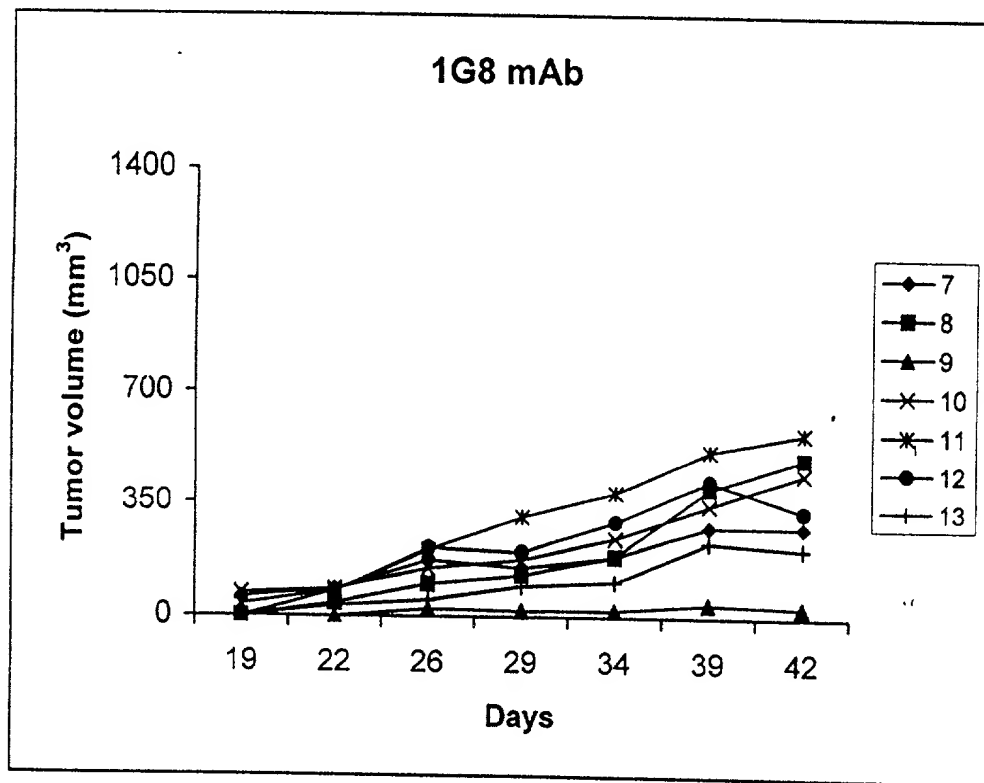
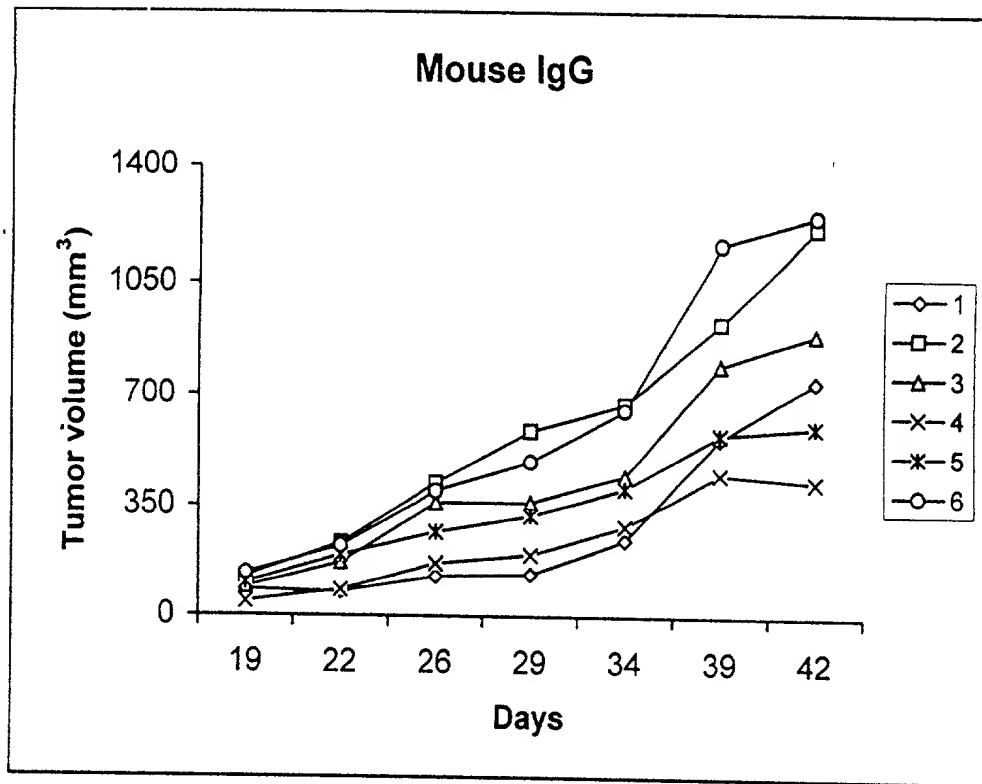


FIG. 55

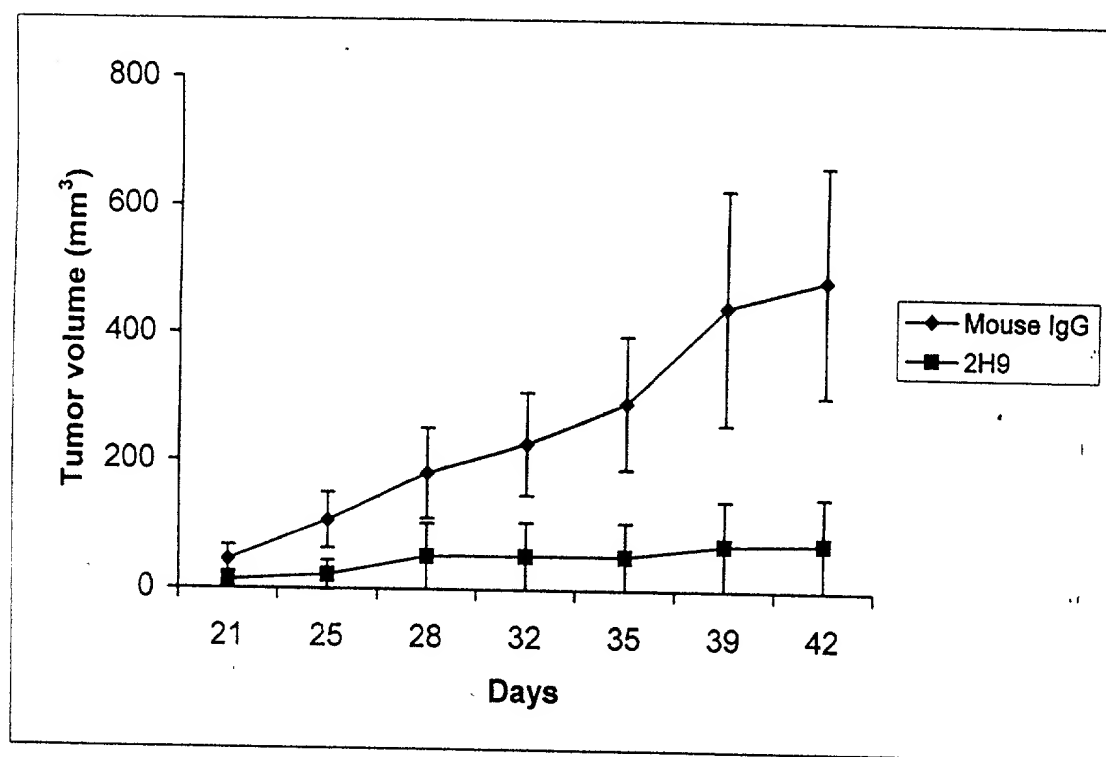
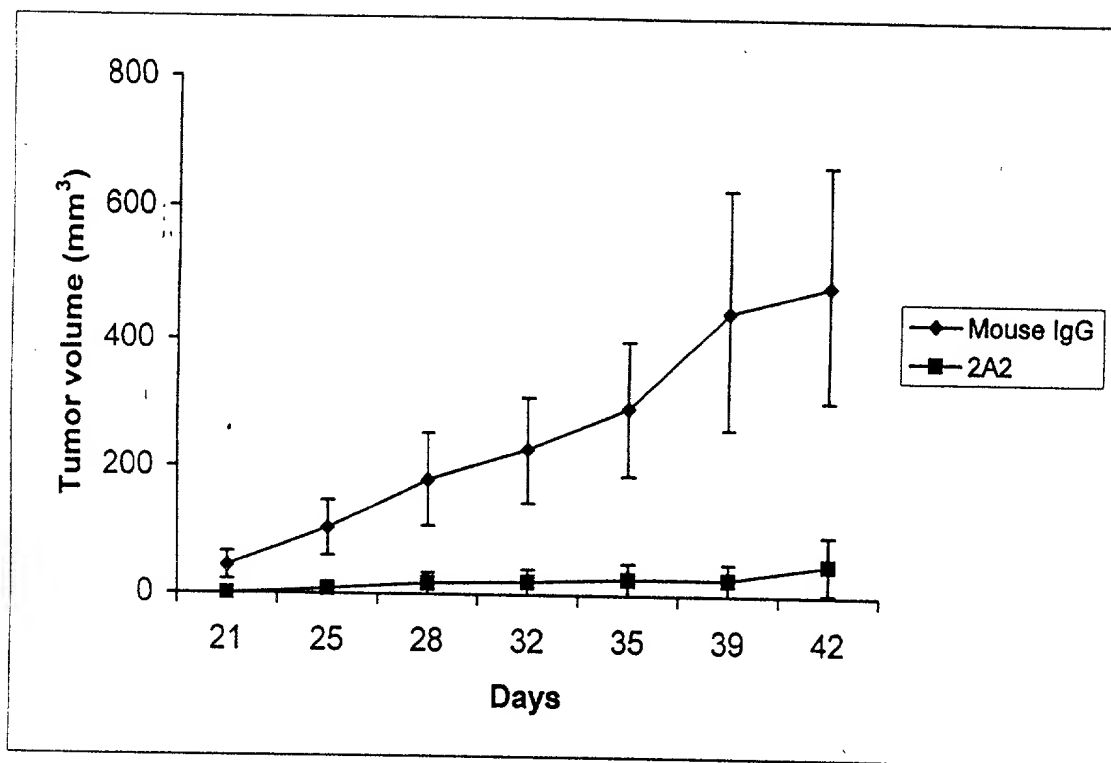


FIG. 56

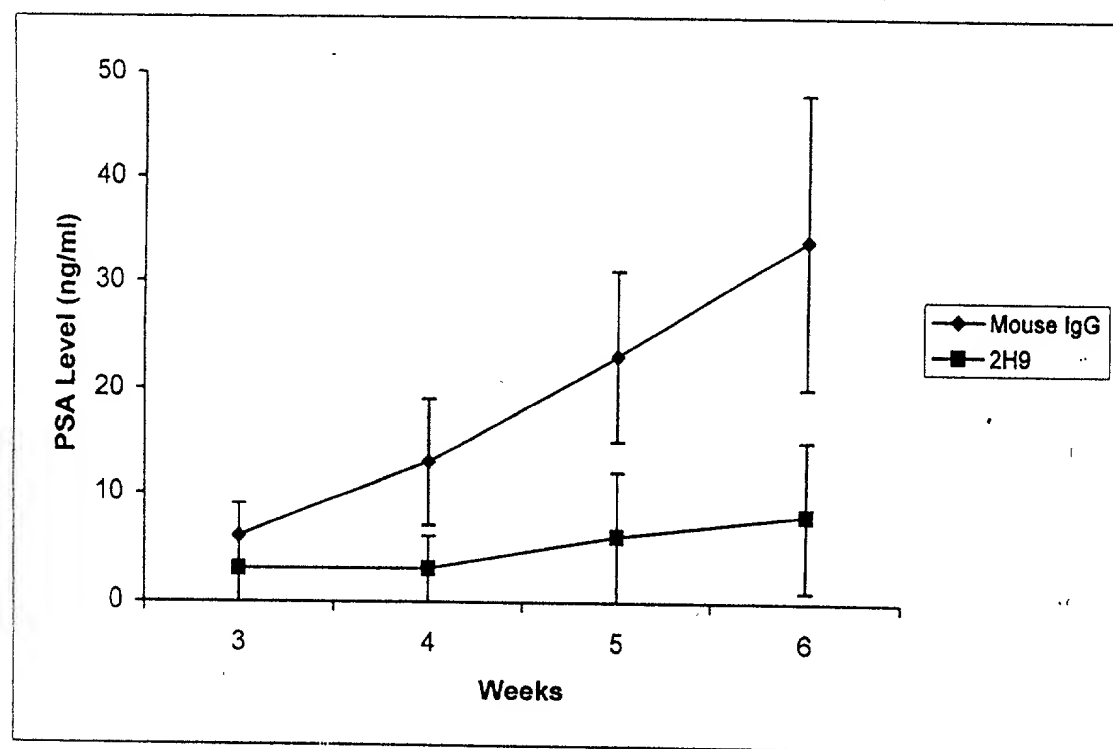
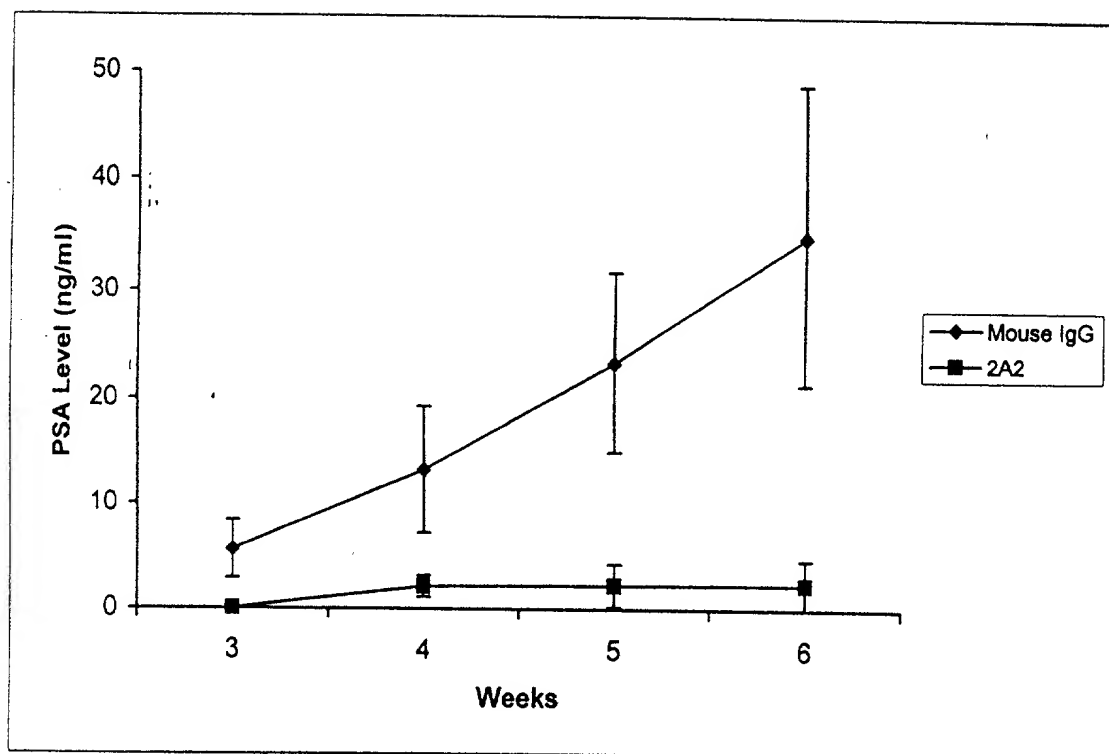


FIG. 57

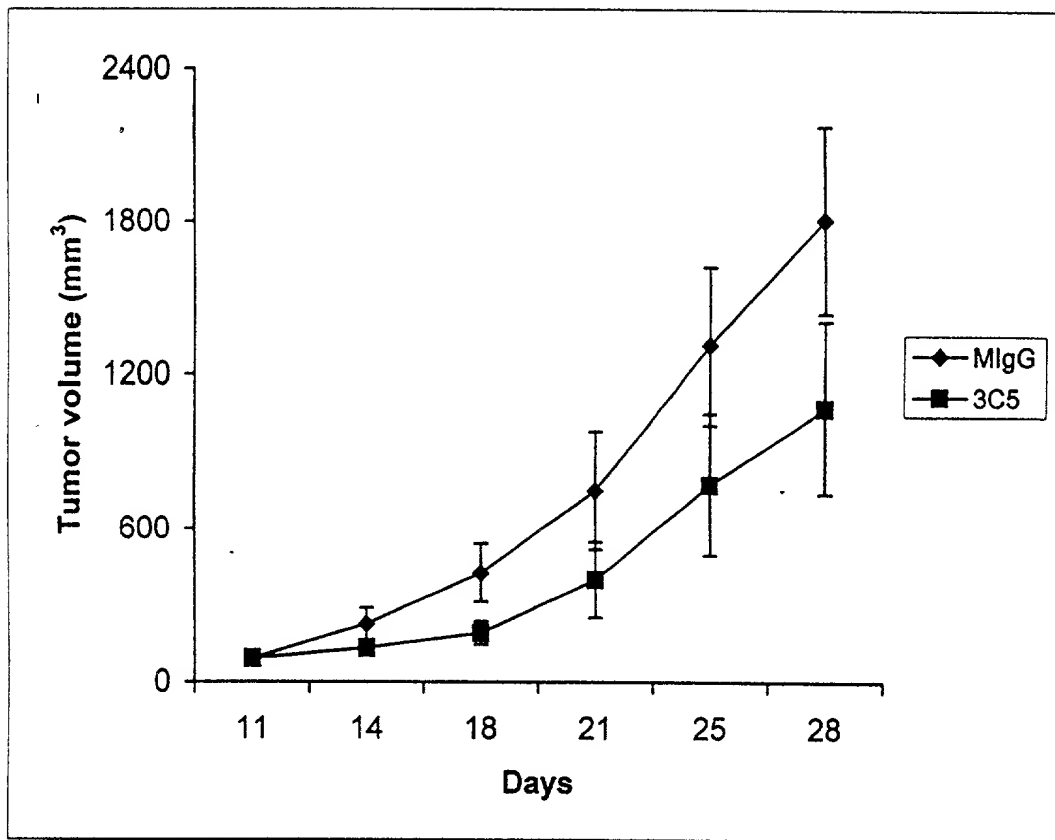


FIG. 58

TGCTTCTTCCTGATGGCAGTGGTTATAGGAGTCAATTCAGAGGTTTCAGCTGCAGCAGTCT 60
C F F L M A V V I G V N S E V Q L Q Q S 20

GGGGCAGAACTTGTGAGGTCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCTGGCTTC 120
G A E L V R S G A S V K L S C T A S G F 40

CDR1
AACATTAAAGACTACTATATACACTGGGTGAATCAGAGGCCTGACCAGGGCCTGGAGTGG 180
N I K D Y Y I H W V N Q R P D Q G L E W 60

CDR2
ATTGGATGGATTGATCCTGAGAATGGTGACACTGAATTTGTCCCGAAGTTCCAGGGCAAG 240
I G W I D P E N G D T E F V P K F O G K 80

GCCACTATGACTGCAGACATTTTCTCCAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300
A T M T A D I F S N T A Y L H L S S L T 100

CDR3
TCTGAAGACACTGCCGTCTATTACTGTAAACGGGGGTTTCTGGGGCCAAGGGACTCTG 360
S E D T A V Y Y C K T G G F W G Q G T L 120

GTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTATCCACTG
V T V S A A K T T P P S V Y P L

FIG. 58

FIG. 59

TTGGTAGCAACAGCCTCAGATGTCCACTCCCAGGTCCAAGTGCAGCAACCTGGGTCTGAA 60
L V A T A S D V H S Q V Q L Q Q P G S E 20

CTGGTGAGGCCTGGAAGTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120
L V R P G T S V K L S C K A S G Y T F S 40
CDR1

AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180
S Y W M H W V K Q R P G Q G L E W I G N 60

ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
I D P G S G Y T N Y A E N L K T K A T L 80
CDR2

ACTGTAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC 300
T V D T S S S T A Y M Q L S S L T S E D 100

TCTGCAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGGATTTGCTTACTGG 360
S A V Y Y C T S R S T M I T T G F A Y W 120
CDR3

GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCTGTCTATCCA 420
G Q G T L V T V S A A T T T A P S V Y P 160

CTGGCC
L A

FIG. 59 "EST-144"

FIG. 60

AATGACTTCGGGTTGAGCTGGGTTTTTATTATTGTTCTTTTAAAAGGGGTCCGGAGTGAA 60
N D F G L S W V F I I V L L K G V R S E 20

GTGAGGCTTGAGGAGTCTGGAGGAGGCTGGGTGCAACCTGGAGGATCCATGAAACTCTCC 120
V R L E E S G G G W V Q P G G S M K L S 40

TGTGTAGCCTCTGGATTACTTTTCAGTAATTACTGGATGACTTGGGTCCGCCAGTCTCCA 180
C V A S G F T F S N Y W M T W V R Q S P 60
CDR1

GAGAAGGGGCTTGAGTGGGTTGCTGAAATTCGATTGAGATCTGAAAATTATGCAACACAT 240
E K G L E W V A E I R L R S E N Y A T H 80
CDR2

TATGCGGAGTCTGTGAAAGGGAAATTCACCATCTCAAGAGATGATTCCAGAAGTCGTCTC 300
Y A E S V K G K F T I S R D D S R S R L 100

TACCTGCAAATGAACAACTTAAGACCTGAAGACAGTGGAATTTATTACTGTACAGATGGT 360
Y L Q M N N L R P E D S G I Y Y C T D G 120

CTGGGACGACCTAACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACA 420
L G R P N W G Q G T L V T V S A A K T T 140
CDR3

CCCCCATCTGTCTATCCACTGGCCCCTTGTGTA
P P S V Y P L A P C V

SEQUENCE SHEET

Overall sample		Non-responders		Responders	
	n		n		n
Age (years)					
Mean	50.0	50.0	50.0		
SD	10.0	10.0	10.0		
Range	20-70	20-70	20-70		
Gender					
Male	100	100	100		
Female	100	100	100		
Marital status					
Married	100	100	100		
Single	100	100	100		
Divorced	100	100	100		
Widowed	100	100	100		
Education					
High school	100	100	100		
College	100	100	100		
Postgraduate	100	100	100		
Occupation					
Manager	100	100	100		
Professional	100	100	100		
Service	100	100	100		
Unemployed	100	100	100		
Retired	100	100	100		
Income (USD)					
Mean	1000	1000	1000		
SD	200	200	200		
Range	500-1500	500-1500	500-1500		
Health status					
Good	100	100	100		
Fair	100	100	100		
Poor	100	100	100		
Smoking status					
Smoker	100	100	100		
Non-smoker	100	100	100		
Alcohol consumption					
Regular	100	100	100		
Occasional	100	100	100		
Never	100	100	100		
Exercise					
Regular	100	100	100		
Occasional	100	100	100		
Never	100	100	100		
Stress level					
High	100	100	100		
Medium	100	100	100		
Low	100	100	100		
Life satisfaction					
High	100	100	100		
Medium	100	100	100		
Low	100	100	100		

1G8 1gG_{1k}

2H9 1gG_{1k}

1G8 1gG_{1k}

2H9 1gG_{1k}

1G8 1gG_{1k}

2H9 1gG_{1k}

FIG. 62

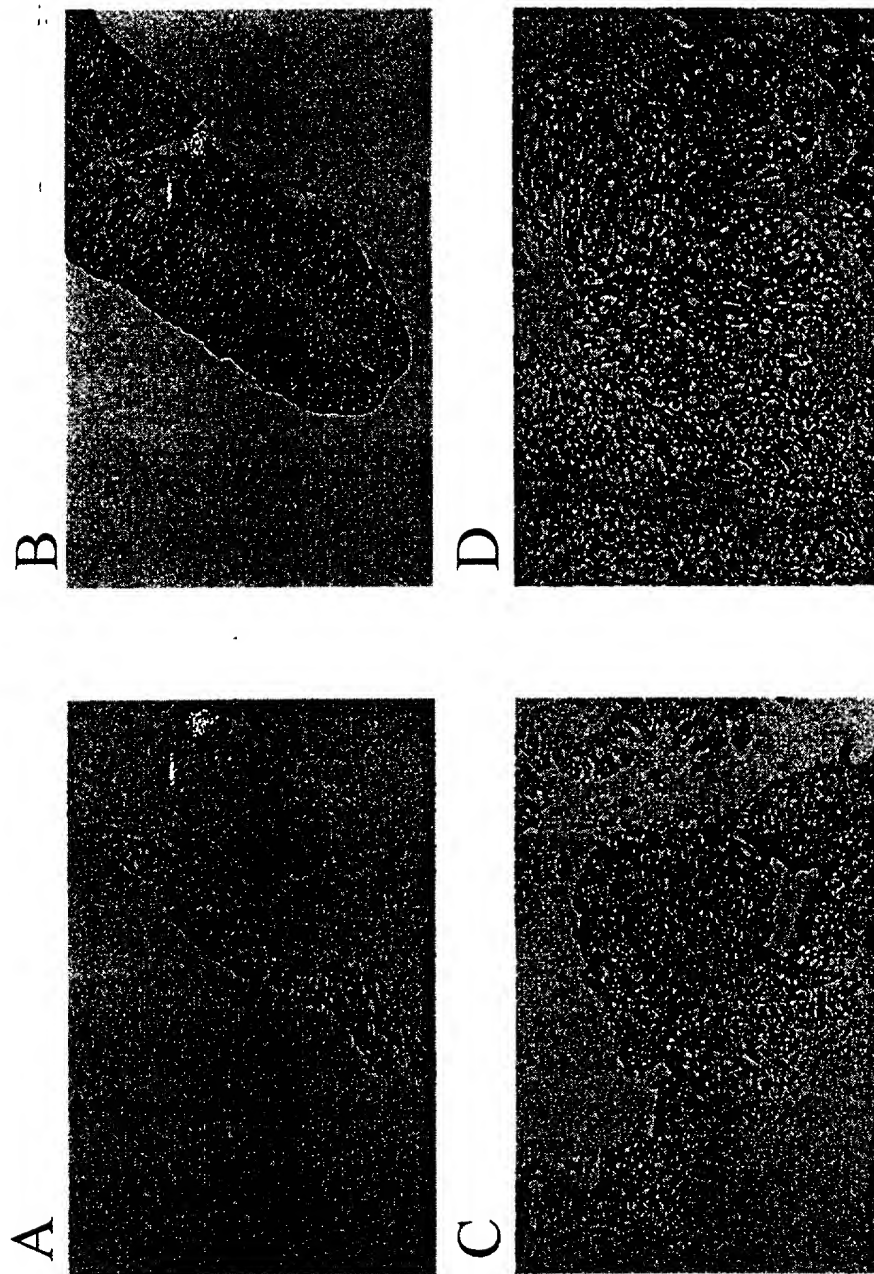


FIG. 63

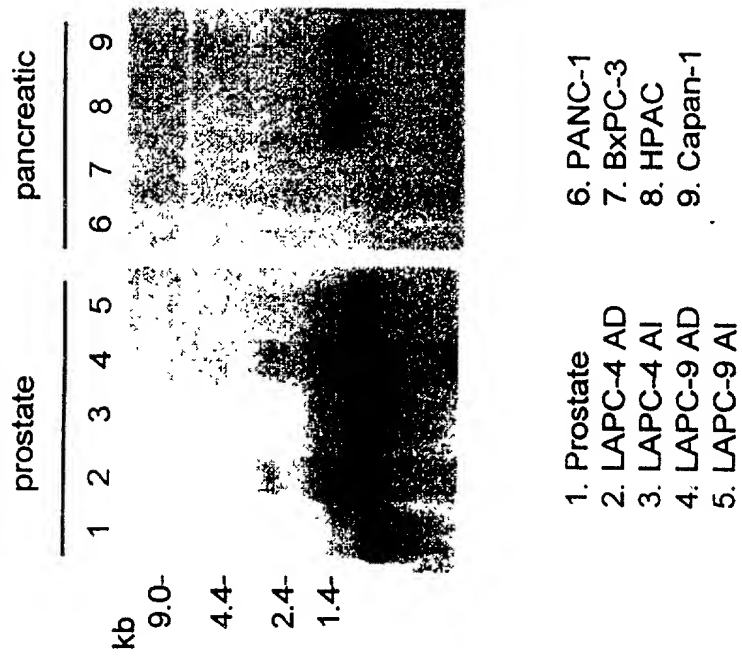
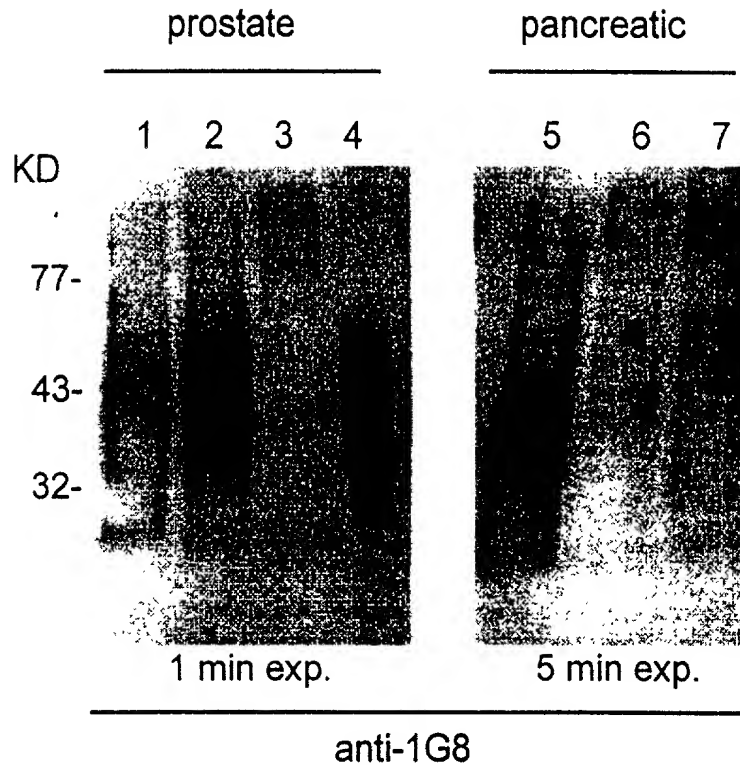


FIG. 64



1. LAPC-4 AD
2. LAPC-9 AI
3. LNCaP
4. LNCaP-PSCA

5. HPAC
6. Capan-1
7. ASPC-1

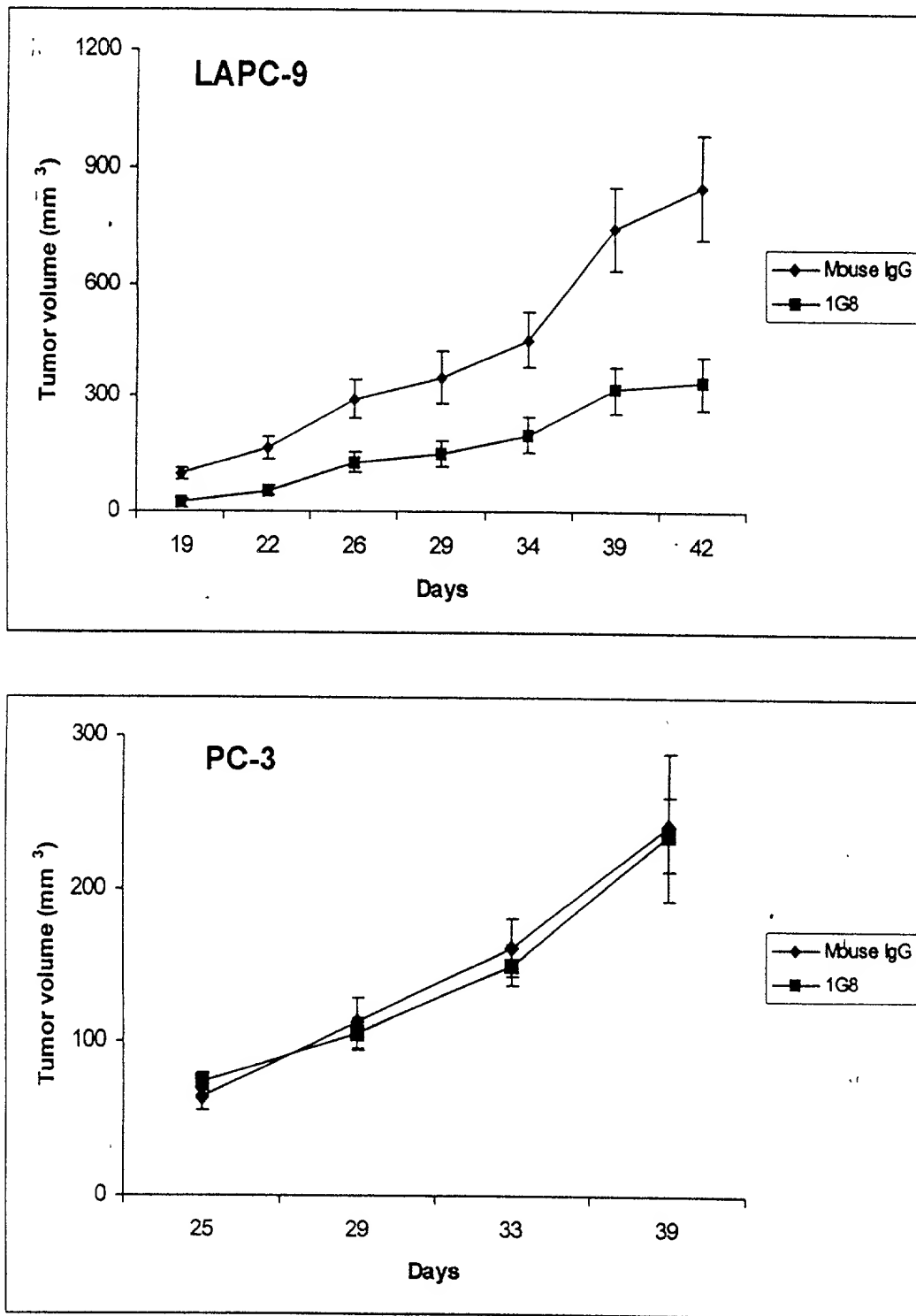
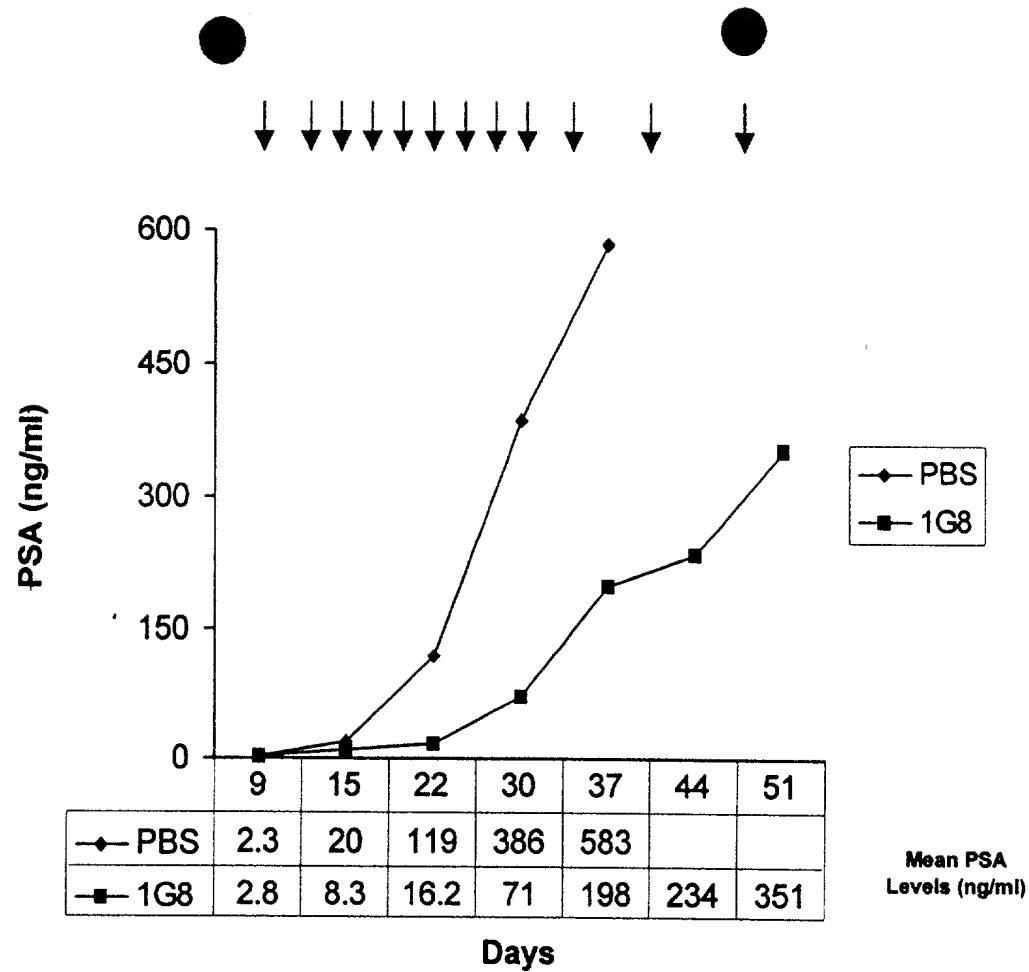


FIGURE 65

TOP SECRET

A)



B)

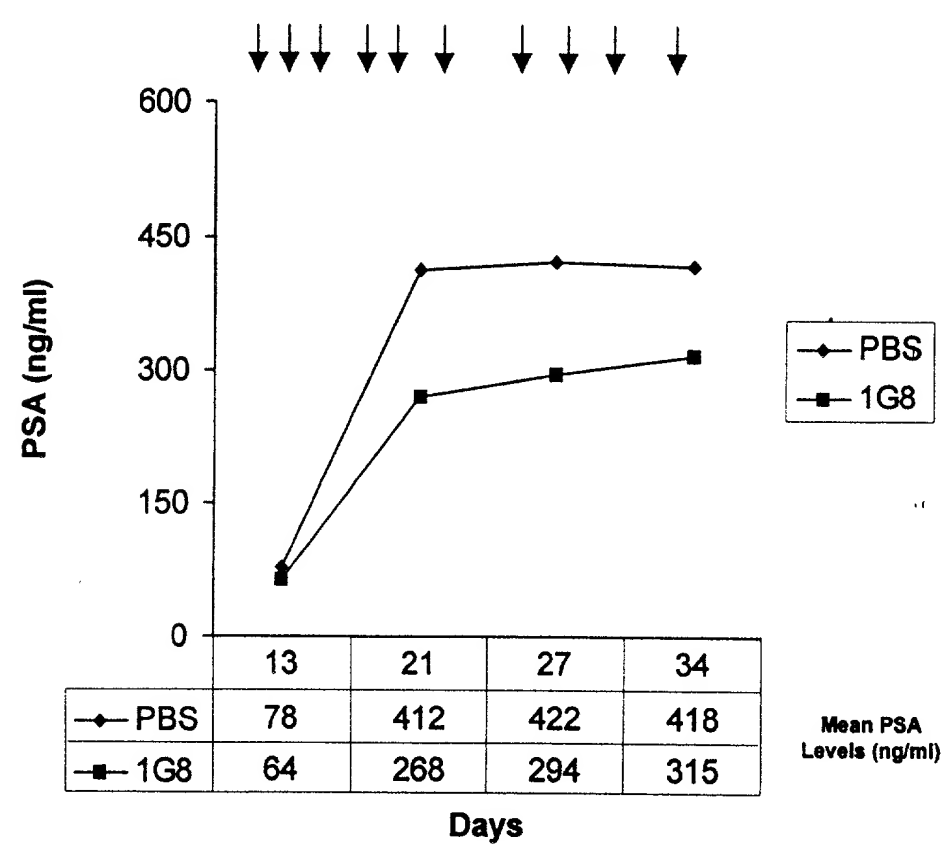
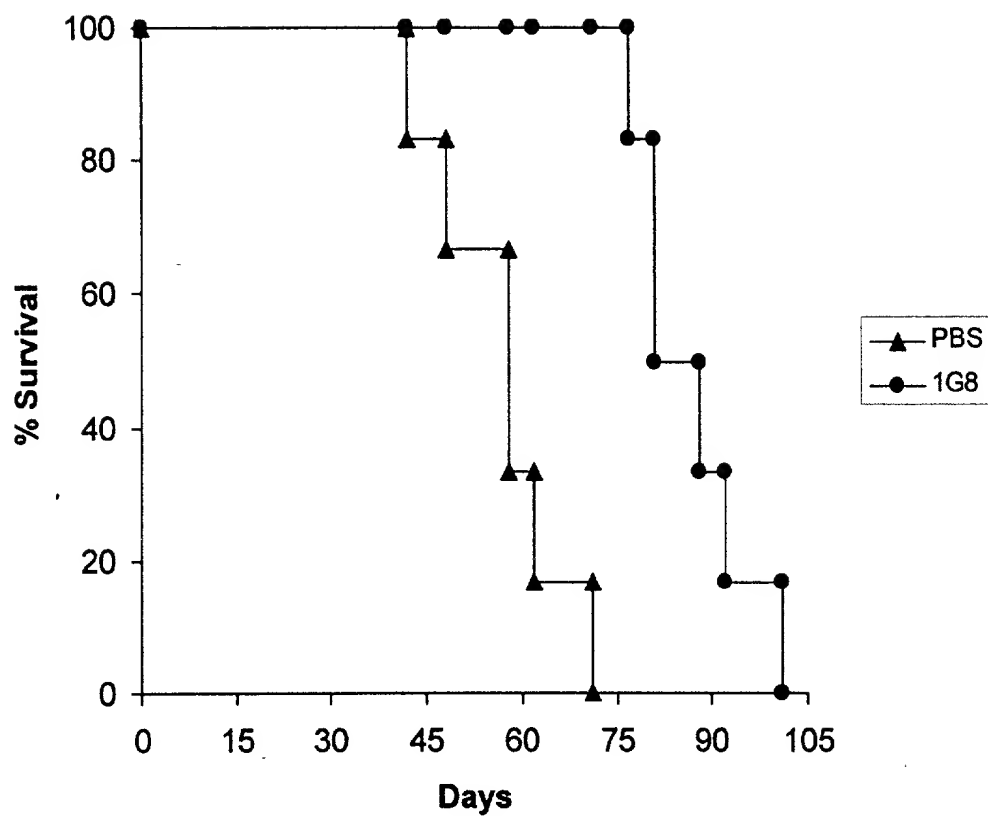


Figure 66

A)



B)

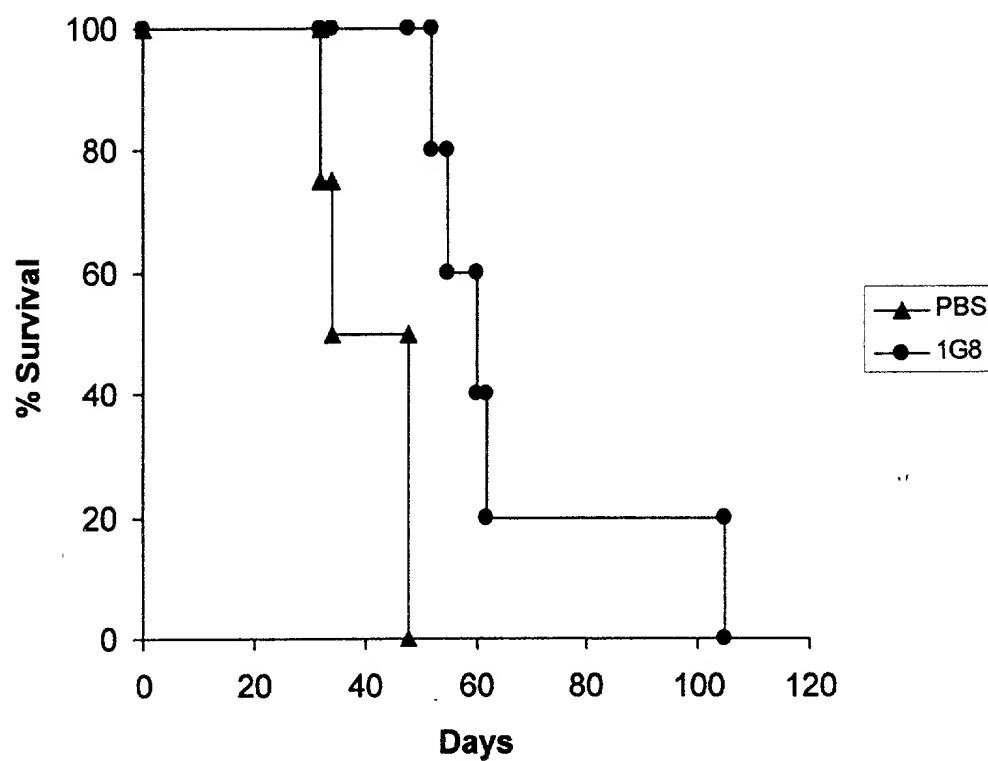
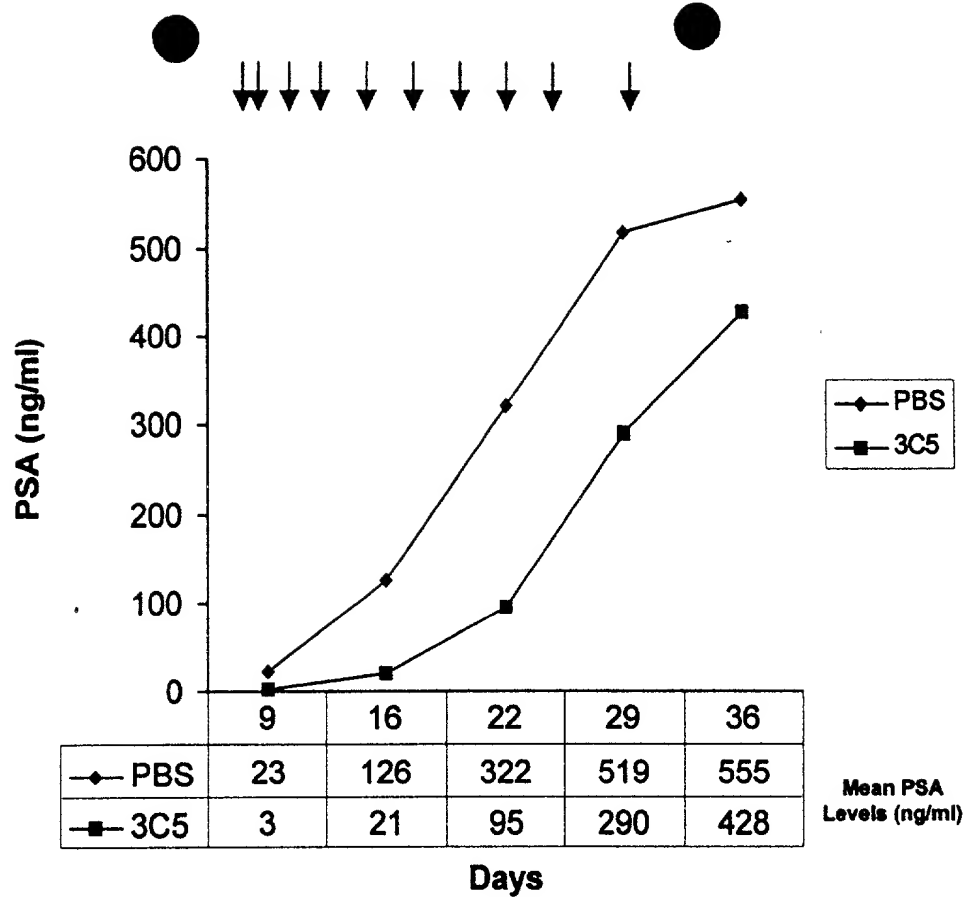


Figure 67

A)



B)

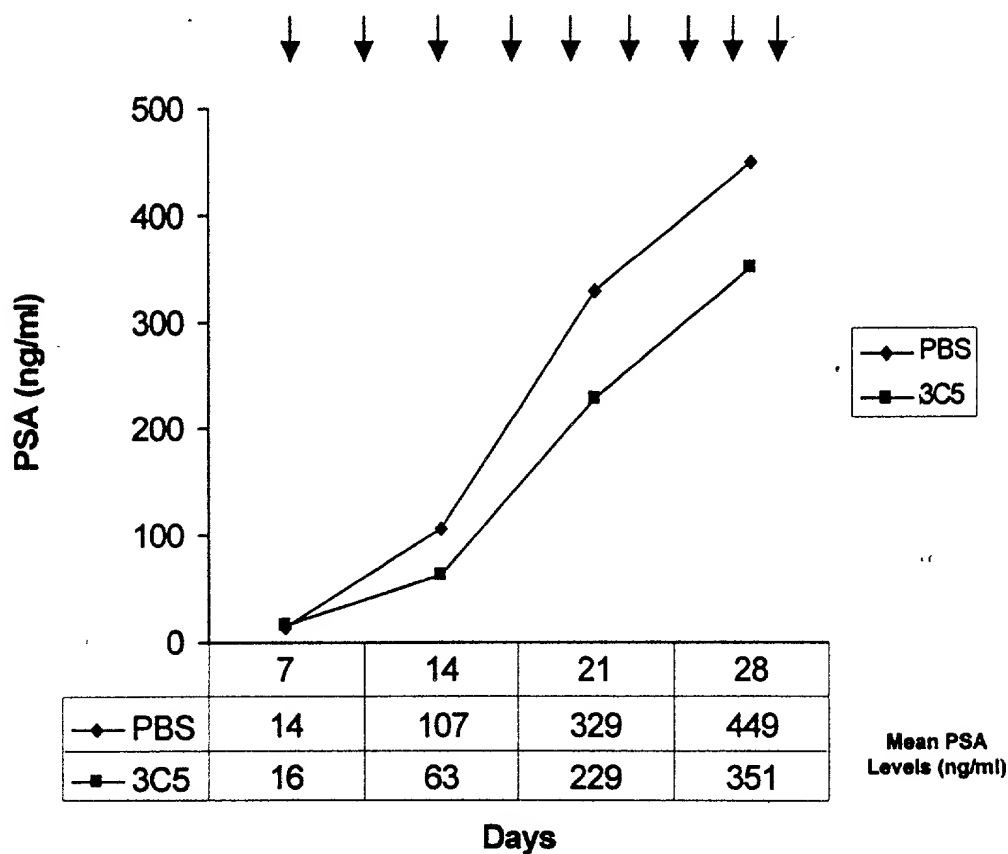
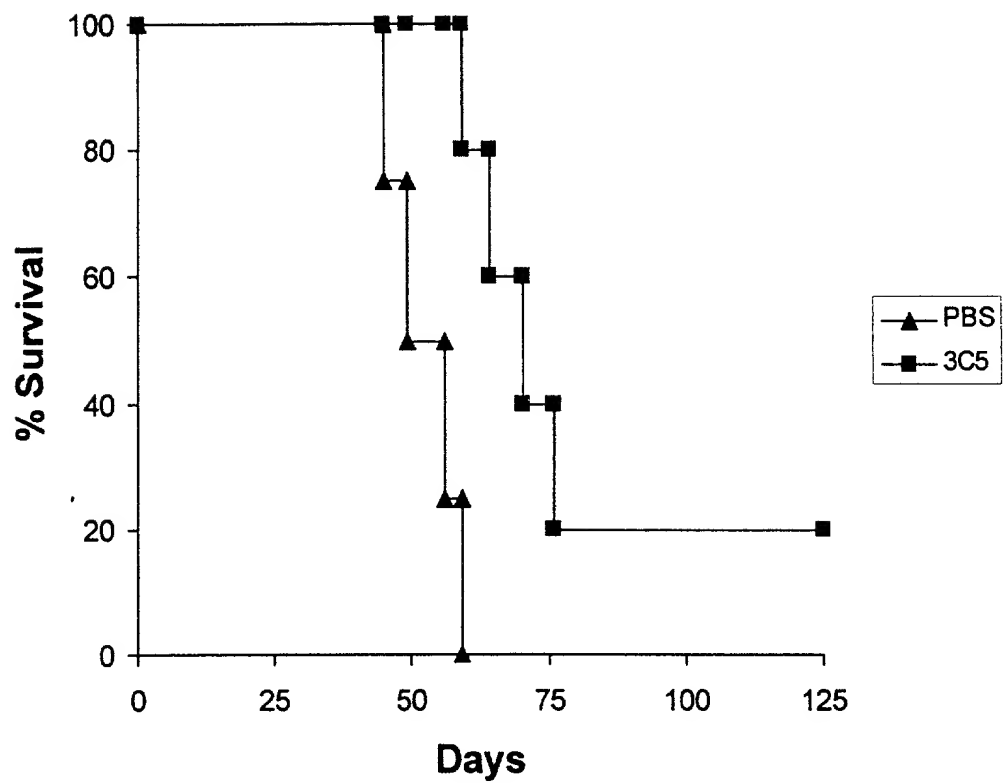


Figure 68

A)



B)

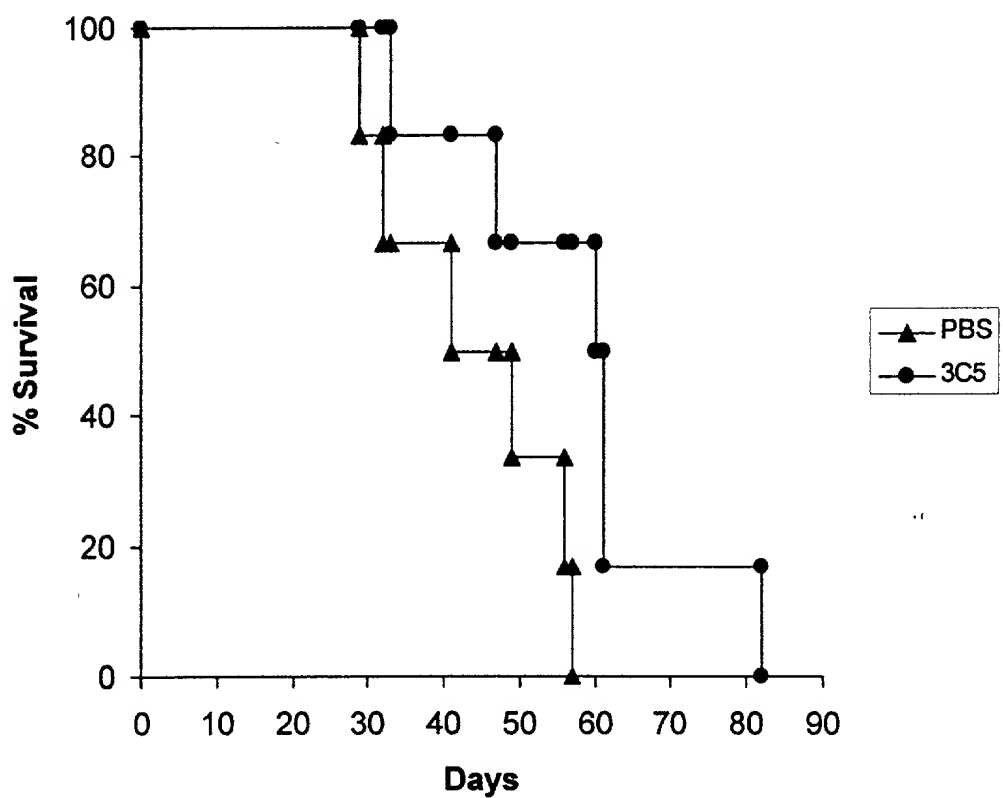


Figure 69

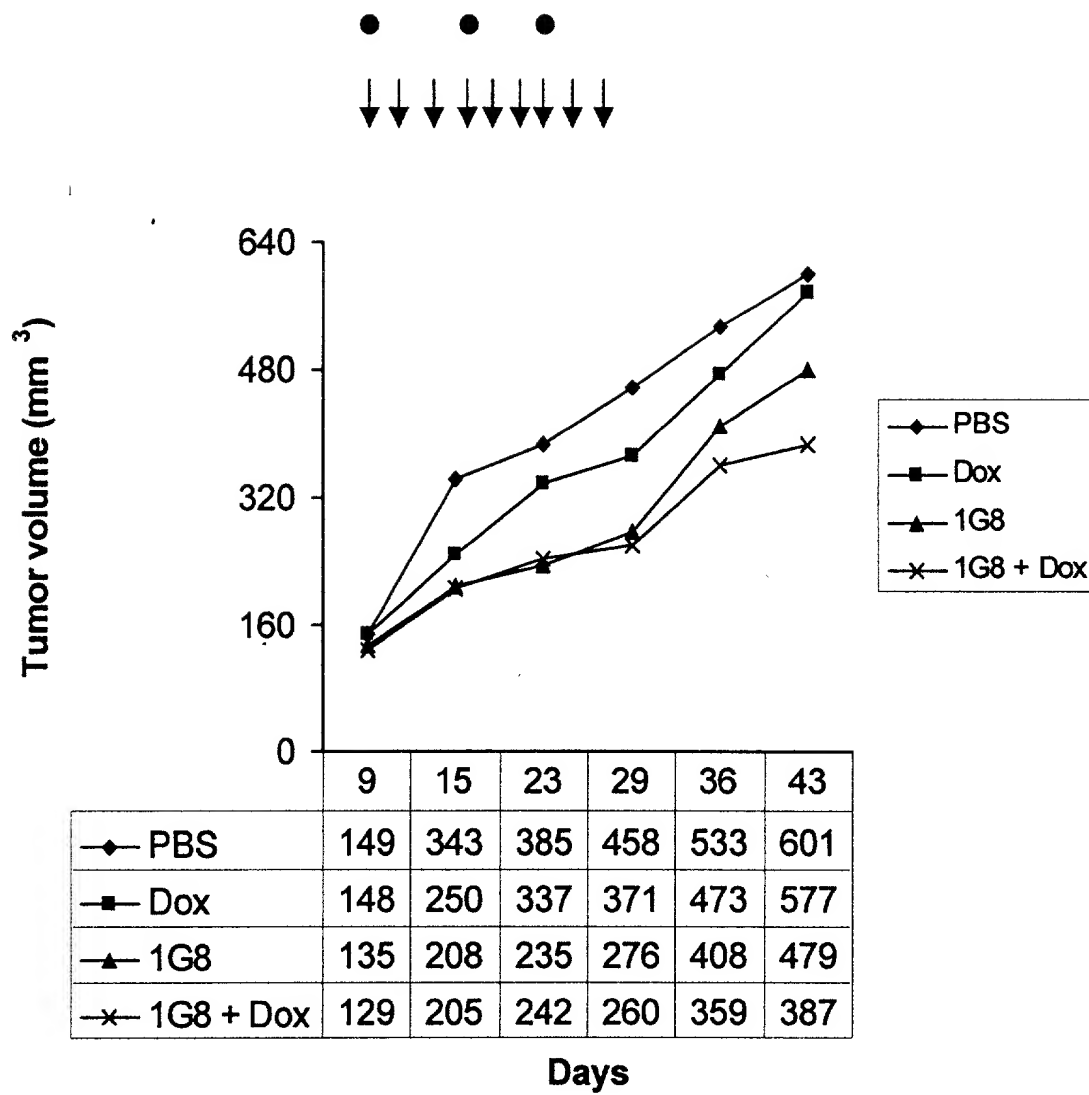
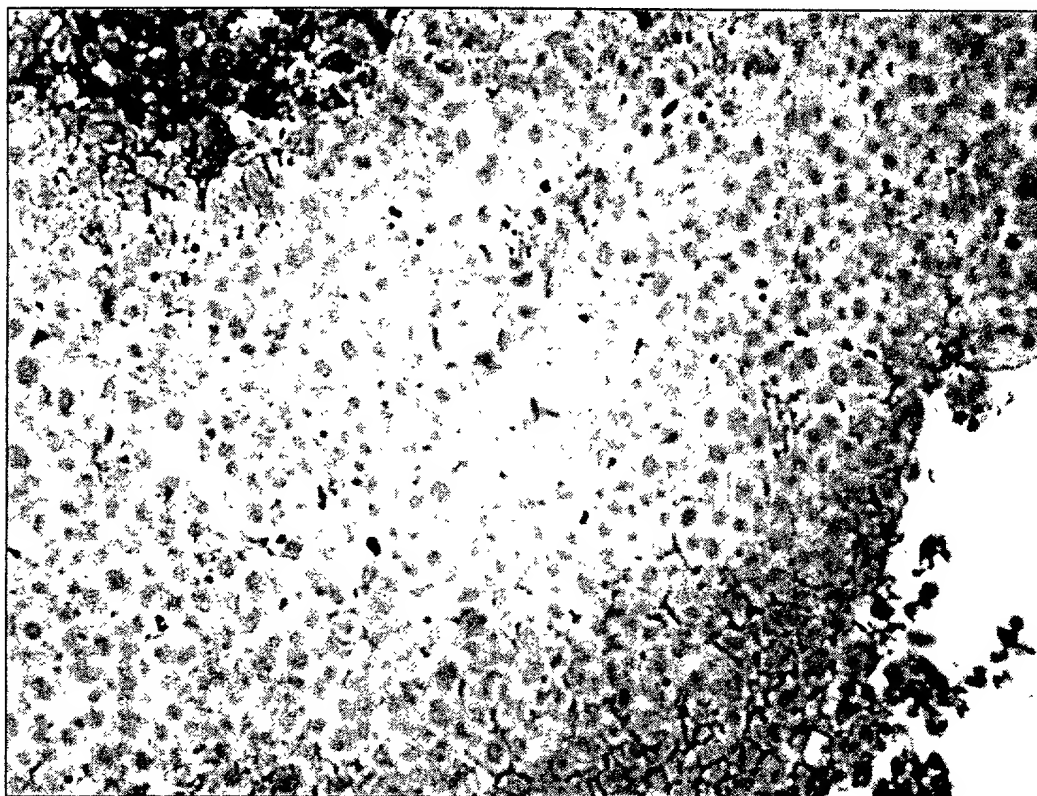


Figure 70

PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

3C5 Treated



mIgG Treated

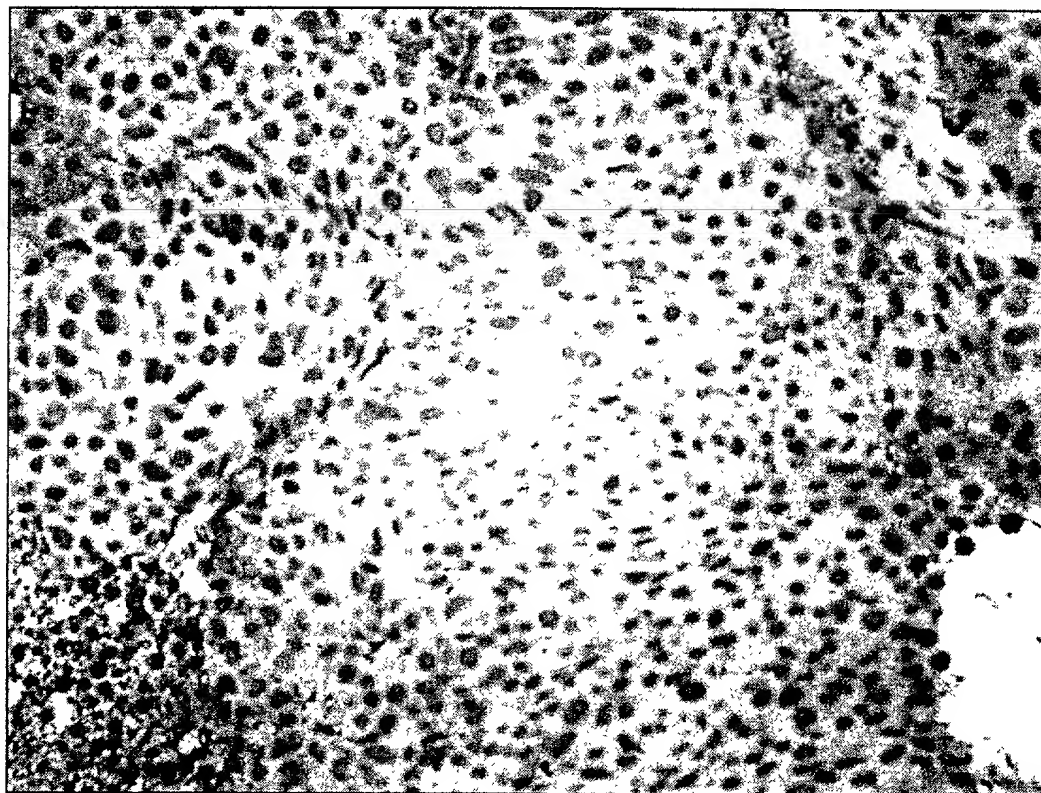


Figure 71

3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors

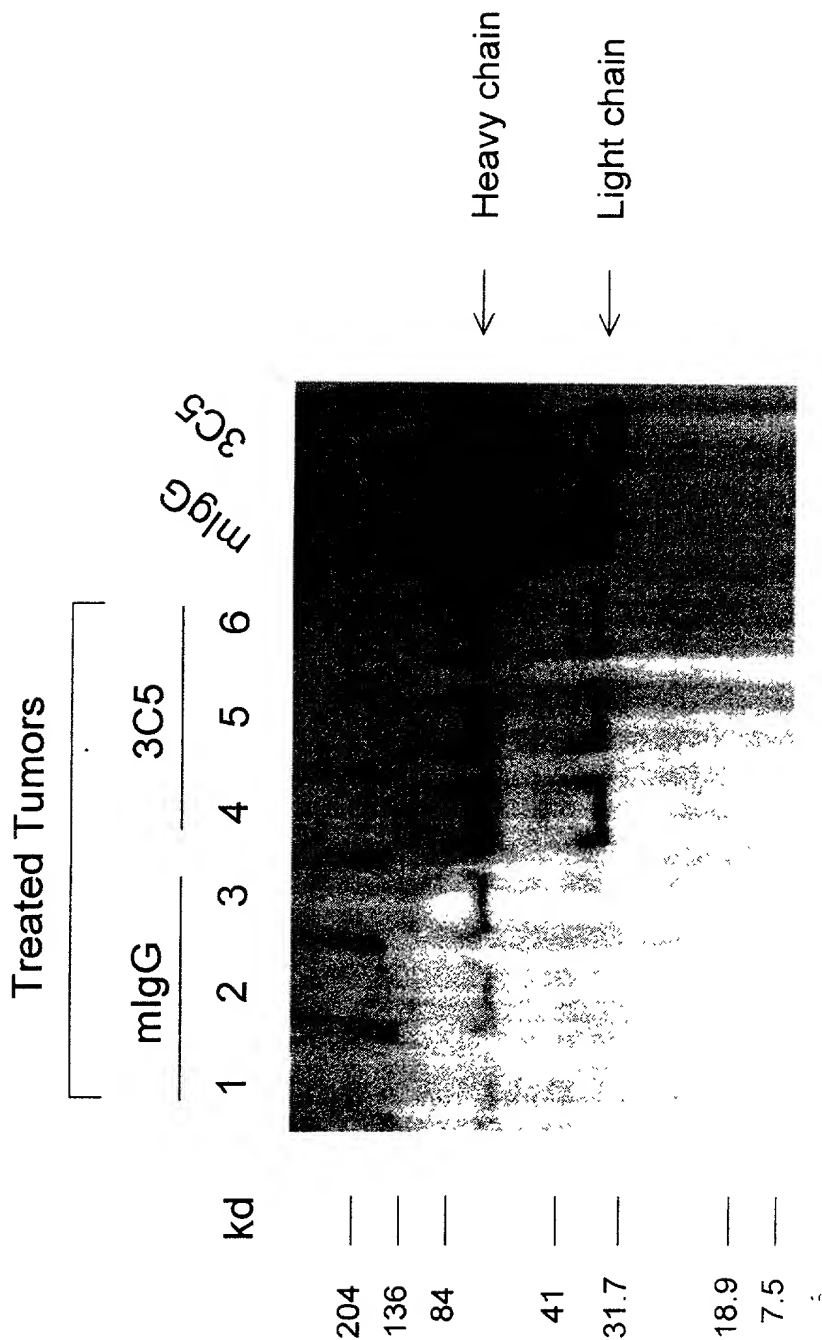
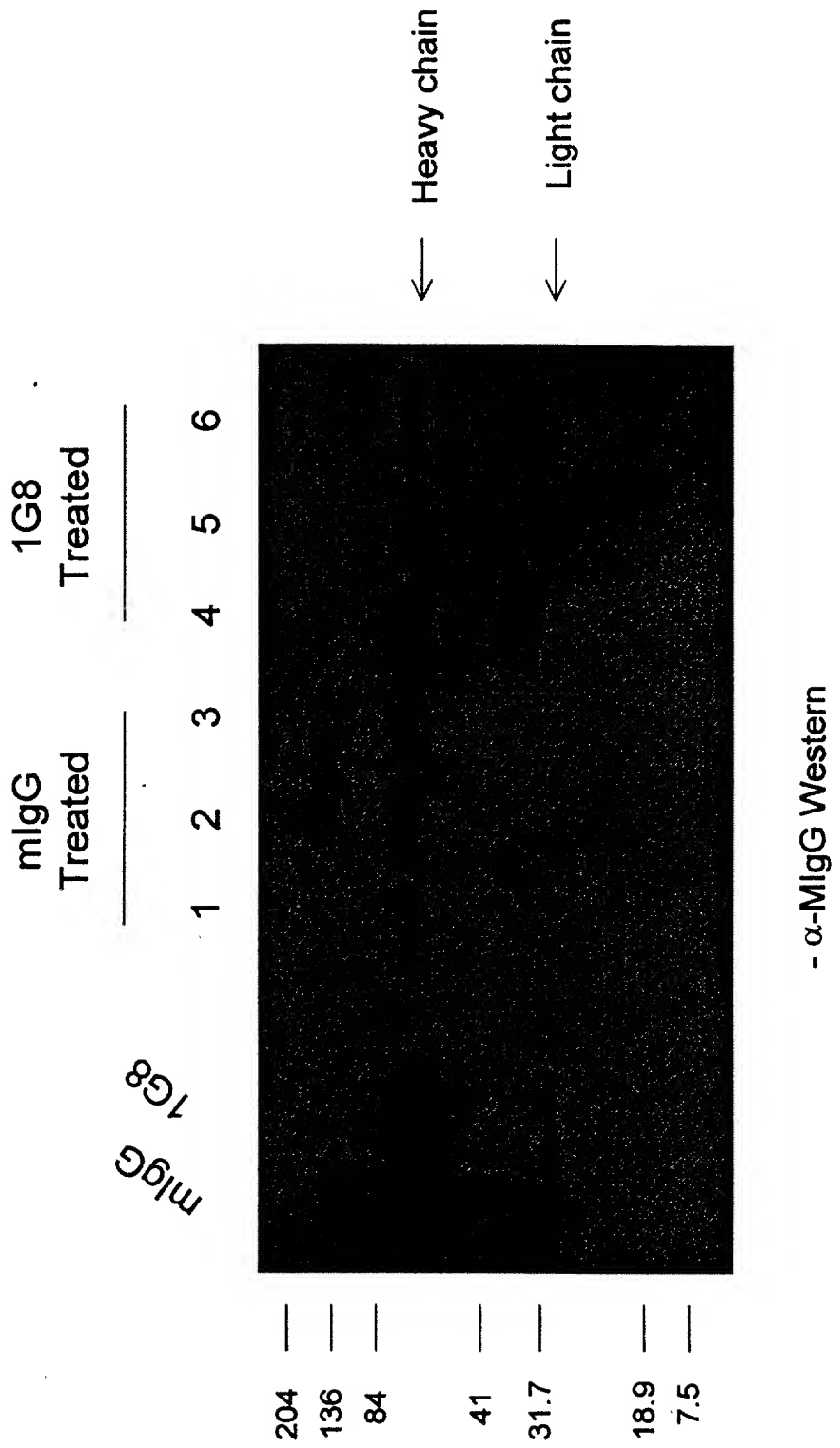


Figure 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAb TO ESTABLISHED LAPC-9 TUMORS



Method: Mice bearing established LAPC-9 tumors (>100 mm³) were injected with either mlgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.

Figure 73